Agroforestry on post-mining restoration: a challenge beyond plant mixture systems

Thèse

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Résumé

L'agroforesterie est un système dynamique d'aménagement écologique des ressources naturelles renouvelables, qui en intégrant les espèces ligneuses aux champs agricoles, fermes et autres paysages, diversifie, augmente la production et engendre des bénéfices socio-économiques et environnementaux. En tant que solution pour la fourniture des services écosystémiques, son application à la restauration des écosystèmes dégradés, endommagés ou détruits devient très importante. Les terres dégradées, endommagées ou détruites (3-D) par l’exploitation minière sont caractérisées par un sol de faible fertilité et parfois des niveaux élevés des contaminants. Ces conditions les rendent difficile l'obtention d'un avantage à court terme de l'agroforesterie en comparaison aux terres arables, mais sa principale fonction restaurative consistant à rétablir les services écosystémiques et à accroître la résilience peut être bénéfique à long terme. Le défi consiste à développer la meilleure stratégie pour accélérer la productivité des plantes tout en améliorant le sol et l’écosystème grâce à une combinaison des techniques d’ingénierie écologique pour la biorestauration des milieux miniers. Nous explorons ici le mélange de plantes, d’inoculation microbienne et d’amendement en biochar, dans un système agroforestier ligneux-herbacé. L’objectif est de trouver le meilleur scénario de biorestauration à partir des effets combinés de mélange de plantes et d’autres facteurs écologiques connexes.

Des recherches antérieures sur l'agroforesterie et la restauration ont été révisées à travers le monde entier, y compris l’application du concept agroforestier en biorestauration des terres post-minières. La stratégie de restauration connue dans un milieu donné ne constitue pas une solution universelle. Ainsi, l'identification de tout aspect important des travaux antérieurs sur la restauration et l'agroforesterie est cruciale. La stratégie de mélange des plantes est un facteur important dans les processus de succession. Dans cette recherche, nous avons appliqué le concept de parcelles de Nelder modifié pour la combinaison d'espèces de plantes dans une expérience en serre sur les stériles et les résidus fins afin d'explorer l'interaction au début de la plantation. Nous avons aussi appliqué l’inoculum microbien et le biochar sur le mélange de plantes dans des essais en serre et sur le terrain sur les stériles et les résidus fins comme matériau de sol d’un site post-extraction de l’or. La performance de la co-plantation de quatre espèces ligneuses (*Alnus viridis* (Chaix) DC. ssp. *crispa* (Aiton) Turrill, *Picea* *glauca* (Moench) Voss, *Populus tremuloides* Michx. et *Salix arbusculoides* Andersson) avec les les espèces de plantes herbacées (*Avena sativa* L., *Festuca rubra* L. *et Trifolium repens* L.) a été évaluée. Le mélange de plantes est un principe très important dans les pratiques de restauration, étant donné son rôle connu pour augmenter la biodiversité et la diversité fonctionnelle dans le système écologique durable. Bien que la stratégie de mélange ait été rarement explorée, nous avons constaté que la combinaison des espèces avait un effet neutre (ni avantages, ni inconvénients) par rapport à une seule espèce dans l'expérience de parcelles de Nelder. En même temps, l'effet positif de la densité suggérait que l'amélioration du microclimat avait joué un rôle dans la croissance précoce des plantations. L'essai sur le terrain confirme l'effet positif de la modification du microclimat sur la productivité des plantes lorsque la densité de plantation est élevée. Le compromis sur la concurrence des plantes a montré que la densité la plus élevée ne constitue pas nécessairement une condition optimale pour la productivité des plantes. L'effet d'interaction du biochar et du traitement d'inoculation montre l'intérêt de ce traitement, mais l'impact varie selon la densité de plantation.

La densité de plantation a été démontrée comme le facteur le plus important pour générer l'effet positif net. Nous suggérons que le mécanisme était corrélé à l'amélioration du microclimat par la conservation de l'eau des plantes du sol et l'amélioration de l'activité microbienne par rapport à la modification de la température du sol. Par conséquent, mettre l'accent sur l'amélioration du microclimat, ainsi que sur d'autres facteurs combinés, y compris l'inoculation microbienne et l'amendement du biochar, est très important pour accélérer les processus de restauration.

Abstract

Agroforestry is a dynamic system of ecological management of renewable natural resources, which by integrating woody species into agricultural fields, farms and other landscapes, diversifies and sustains production for increased socio-economic and environmental benefits. As a solution for the provision of ecosystem services, its application to the restoration of degraded damaged, or destroyed ecosystems becomes very important. Degraded, damaged, or destroyed (3-D) lands by mining is characterized by low fertility soil and sometimes high levels of contaminants. These conditions make them difficult to obtain a short-term advantage from agroforestry compared to arable lands, but its main restorative function of restoring ecosystem services and increasing resilience can be beneficial in the long term. The challenge is to develop the best strategy to accelerate plant productivity while improving the soil and the ecosystem through a combination of ecological engineering techniques for bioremediation of mining areas. Here we explore the mixture of plants, microbial inoculation, and biochar amendment, in a woody-herbaceous agroforestry system. The goal is to find the best bioremediation scenario from the combined effects of mixing plants and other related ecological factors.

Previous research on agroforestry and restoration has been reviewed worldwide, including the application of the agroforestry concept in bioremediation of post-mining land area. The known restoration strategy in a given environment is not a universal solution. Thus, the identification of any important aspect of previous work on restoration and agroforestry is crucial. The strategy of mixing plants is an important factor in the successional process. But a statistical accounting of plant-plant interactions and adaptation to multi-species conditions is hard to achieve in field experiments; trials under controlled conditions can distinguish effects of planting density and species interactions in the early stages of plant establishment. In this research, we applied the concept of modified Nelder plots for the combination of plant species in a greenhouse experiment on waste rock and fine tailing to explore the interaction at the start of planting. We also applied microbial inoculum and biochar to the plant mixture in greenhouse and field tests on waste rock and fine tailing as soil material on a post-gold mining site. The performance of the co-planting of four woody species: green alder (*Alnus viridis* (Chaix) DC. ssp. *crispa* (Aiton) Turrill); white spruce (*Picea glauca* (Moench) Voss);

trembling aspen (*Populus tremuloides* Michaux); and littletree willow (*Salix arbusculoides* Andersson) with the herbaceous plant species: oat (*Avena sativa* L.); red fescue (*Festuca rubra* L.) and white clover (*Trifolium repens* L.) was evaluated. Mixing plants is a very important principle in restoration practices, given its known role to increase biodiversity and functional diversity in the sustainable ecological system. Although the plant mixing strategy has been rarely explored, we have found no mixture provided advantages for both species in paired combinations. At the same time, the positive effect of the density on plant growth suggested that the microclimate improvement had played a role in the early growth of the plantations. The field trial confirms the positive effect of the microclimate modification on plant productivity in higher planting density. The trade-off on plant competition has shown, however, that the highest density does not necessarily show an optimal condition for plant productivity. The interaction effect of biochar and inoculation treatment shows the benefit of this treatment, although the impact varies according to the density of planting.

The plantation density was shown as the most important factor in generating the net positive effect. We suggest that the mechanism was correlated with the microclimate improvement through soil plant water conservation and microbial activity enhancement over soil temperature modification. Hence, putting emphasis on microclimate improvement, along with other combined factors including microbial inoculation and biochar amendment is very important for accelerating the restoration processes.

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List of abbreviations

AC	*Alnus viridis* subsp. *crispa*
PG	*Picea glauca*
PT	*Populus tremuloides*
RCE	Relative Competition Effect
RSE	Residual Standard Error
SA	*Salix arbusculoides*
SLA	Specific Leaf Area

For my parents, my wife and the whole family
in Indonesia

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Foreword

This thesis is written in English. It contains a general introduction and a general conclusion; and three chapters as scientific articles. The first chapter will be submitted to the journal of Restoration Ecology (ISSN: 1526-100X), the second chapter was submitted to Canadian Journal of Forest Research (ISSN:0045-5067) and the third chapter will be submitted to the journal of Plant and Soil (ISSN: 1573-5036).

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General Introduction

The industrial era has led to land degradation and depletion of natural resources with some negative impacts on the environment. Modern, energy-dependent economies cannot thrive without the exploitation of natural resources. However, natural landscapes cannot survive without stewardship and proper management. Thus, ecological restoration has been a common interest among world leaders and the society at large. Indeed, the United Nations (UN) General Assembly declared on 1 March 2020 the UN Decade on Ecosystem Restoration (2021-2030) with the aim to massively scale up the restoration of degraded and destroyed ecosystems as a proven measure to fight the climate crisis and poverty, and enhance food security, water supply and biodiversity. The definition of "restoration" has been changing from rebuilding the ecosystems using the past reference into the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed (Clewell *et al.*, 2004). The previous objective is believed to be too idealistic and may no longer applicable on current era of the changing climate (Choi *et al.*, 2008; Suding, 2011). Therefore, forward-looking paradigms which focusing on enhancing the ecosystem services and increasing the resilience to future changes was suggested for current ecological restoration practices (Choi *et al.*, 2008; Naeem, 2006; Suding, 2011).

When the land is disturbed by surface mining operations, the terms remediation, reclamation, restoration and rehabilitation (R4) are commonly used interchangeably or otherwise vaguely defined, which is difficult to comprehend by regulators, industry, environmental practitioners, local communities and the general public (Lima *et al.*, 2016). The latter stand to benefit from a precise terminology based on agreed-upon end-goals. These definitions range from the avoidance of exposure to pollutants (remediation) to the full recovery of the original ecosystem (restoration). The definitions and approaches of reclamation and rehabilitation with surface mining legacies may often overlap and aims to recover key ecosystem services and biogeochemical functions within a replacement ecosystem or rehabilitation, which implies a repurposing of the landscape (Lima *et al.*, 2016).

Agroforestry system can be an option for ecological restoration according to recent definition by Society for Ecological Restoration (Clewell *et al.*, 2004). Several definitions have been proposed to agroforestry and the most recent definition is by Leakey (1996) who defines agroforestry as agroforestry as ‘a dynamic, ecologically based, natural resource management system that, through the integration of trees in farm and rangeland, diversifies and sustains production for increased social, economic and environmental benefits’. Being one of the solutions for future increment on land requirement and food demands, its application on land restoration is very important. The application of agroforestry systems for land restoration can be an ideal solution for both natural resources and ecosystem improvement (Atangana *et al.*, 2014; Garrity, 2012; Vieira *et al.*, 2009).

The degraded land is estimated about half of world land area and is still increasing (Gibbs and Salmon, 2015). While agroforestry could be an ideal practice for restoration, degraded land usually has poor soil condition and will not be suitable for agriculture in short term. Nevertheless, agroforestry application on degraded land is still possible with an intensive management using phytobial remediation technologies that can ameliorate degraded soils, extract, degrade, stabilize and/or store heavy metals and other pollutants (Atangana *et al.*, 2014; Cooper *et al.*, 1996). Agro-succession is one of the method introduced for ecological restoration incorporating agriculture as a transitional phase (Vieira *et al.*, 2009). This method has been successfully applied in South America (Vieira *et al.*, 2009). The forest restoration project in Gunung Kidul - Java, Indonesia, has successfully recovered a highly degraded land through agroforestry by involving the local communities within 30 years (Appanah *et al.*, 2016). The similar agroforestry application under the term “permaculture” has turned severely-degraded dessert land into a fertile garden in Jordan (Hathaway, 2016).

The restoration method somehow cannot be one fit all solution. Its application on post-mining waste land will not be as simple as on the other types of degraded land. The challenges with restoration on degraded post-mining land are the low productivity of soil and high potential contaminants (Markham *et al.*, 2011; Young *et al.*, 2013). The lack of micro and macronutrients on the mining residues make them challenging materials for revegetation. Bioremediation with soil microbial introduction is an option for remediating the soil while increasing the plant uptake capacity of soil nutrients (Vidali, 2001; Vosátka *et al.*, 2006).

The introduction of soil microbes should be followed by the plantation strategy. The plants are hosts for symbiotic microorganisms. Their existence and function are very important for the ecosystem (van der Heijden *et al.*, 1998). The agroforestry principle with species mixtures is expected to provide symbiotic mutualism and provides services required for sustainable ecosystem (Atangana *et al.*, 2014; Ong *et al.*, 2015). The system formed by different functional groups make it more resilient for accelerated recovery and successional processes following disturbances (Choi *et al.*, 2008; Leakey, 2012; Solbrig, 1994). The multi-species approach has been suggested in restoration practices (Lamb, 2011; Martínez-Garza and Howe, 2003; Palmer *et al.*, 2006, 1997). However, the mechanism on how the species diversity and the ecosystem function are very complex (Swift *et al.*, 2004).

Chapter One of this thesis explores the aspects related to the challenge on ecosystem restoration related to phytoremediation and how agroforestry can help in ecological restoration of degraded or destroyed ecosystems. The terms restoration, rehabilitation, remediation, reclamation, revegetation, rewilding, and reforestation (RE7) are vaguely defined, which is difficult to comprehend by regulators, industry, environmental practitioners, local communities and the general public. This chapter thrives to provide clear definitions of these terms and introduce the concept of agroforestry for the ecological restoration of mining sites. Robust science in agroforestry is based on interactions between woody and non-woody components notably the woody and herbaceous species that aim to optimize primary production while increasing functional diversity and the resilience of agroforestry systems.

Chapter Two explores the effect of the species combination of four native woody species green alder (*Alnus viridis* (Chaix) DC. ssp. *crispa* (Aiton) Turrill); white spruce (*Picea glauca* (Moench) Voss); trembling aspen (*Populus tremuloides* Michaux); and littletree willow (*Salix arbusculoides* Andersson) with different growth form and successional type status. One method to explore the species interaction is Nelder wheel plots design. This experimental design aims to test multiple tree spacings in a single plot (Nelder, 1962). The Nelder wheel design is circular plot with concentric rings radiating outward and the spokes connecting the center with the furthest ring (Nelder, 1962; Parrott *et al.*, 2012). While the original method was developed for single species, it can also be applied on mixed species with some modification (Cole and Newton, 1986; Doran *et al.*, 2001; Wurtz, 1995). Its application on the initial plantation on post-mining restoration is expected to give a view on the best strategy for exhibiting the net positive interaction effect between and within species.

The objectives were to find out the effect of mixing species along a gradient density on early growth plantation in post-mining waste soil. The research questions were: 1) is there any advantages of mixing species over monocultural plantation? 2) is the effect consistent along a gradient density? and 3) is there any trade-off between competition and facilitation?

Generally, degraded post-mining soils are physically, chemically, biologically, and mechanically deficient with low levels of organic matter and nutrients, as well as high heavy metal concentrations. Fertilization and soil amendment could be a sustainable option supporting the plant growth, as it has been shown improved soil properties and functions relevant to agronomic and environmental performance (Joseph and Lehmann, 2009; Woolf *et al.*, 2010). 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 hold key roles in sustaining soil health and functioning, are directly affected by the addition of biochar to soils (Ogawa, 1994; Rondon *et al.*, 2007; Steiner, 2008; Warnock *et al.*, 2007). The use of fertilization and large amounts of organic amendments may help the plants to grow better, but it is very costly and may not be sustainable in the long term (Nadeau *et al.*, 2016).

Chapter Three aims to find the method to combine the important factors mentioned above to accelerate the restoration processes. The field trial and greenhouse experiments evaluated the mixture of woody and herbaceous plant species with the introduction of microsymbionts through inoculation and the application of biochar amendments. The spacing effect also helped find out the interaction mechanism between the plant species and their microenvironment.

Chapter 1 Agroforestry on degraded post-mining sites: restoration, rehabilitation, remediation, reclamation, revegetation, rewilding, and reforestation (RE7)

1.1 Résumé

La restauration écologique pouvant regrouper plusieurs concepts RE7 est un processus dynamique visant à rétablir et/ou améliorer les services écosystémiques des terres dégradées, endommagées ou détruites (3D), tout en augmentant la résilience de celles-ci aux changements globaux. En fonction de l'objectifs visés, les pratiques agroforestières sur les terres 3D sont des outils pour restaurer, réhabiliter, réassainir, remettre en état, revégétaliser, ré-ensauvager et reboiser (RE7) en vue de rétablir les services écosystémiques (services d'approvisionnement, de régulation, de soutien et culturels) des terres 3D. Les définitions de ces termes RE7 parfois interchangeables seront précisées mais le terme "réhabilitation/ restauration ou parfois appelé remise en état" est plus généralement utilisé pour les sites miniers et post-miniers industriels. Nous introduisons le concept d'agroforesterie pour la restauration écologique des sites miniers qui est en quelque sorte plus proche de la réhabilitation écologique qui vise à retrouver le fonctionnement initial de l'écosystème avant l'exploitation minière. La science robuste sur l'agroforesterie repose sur les interactions des composantes ligneuses et non ligneuses notamment les espèces ligneuses et herbeuses en vue d'optimiser la production primaire tout en augmentant la diversité fonctionnelle et la résilience des systèmes agroforestiers (SAF). Avec tous leurs avantages, les SAF peuvent être une option pour la restauration écologique de 1 à plus de 6 milliards ha de terre actuellement dégradée sur la terre.

1.2 Abstract

Ecological restoration, which can bring together several RE7 concepts, is a dynamic process aimed at restoring and / or improving the ecosystem services of degraded, damaged or destroyed (3D) lands, while increasing their resilience to global changes. Depending on the objectives, agroforestry practices on 3D lands are tools for restoring, rehabilitating, remediating, reclaiming, revegetating, rewilding and reforesting (RE7) with the aim to re-establish ecosystem services (provisioning, regulating, supporting, and cultural services).

The definitions of these RE7 terms occasionally interchangeable. But the generic term "rehabilitation/restoration" or "reclamation" is more generally used for mined lands or post-industrial sites. The concept of agroforestry for the ecological restoration of mining sites which is closer to ecological rehabilitation which aims to restore the initial functioning of the ecosystem before mining. Robust science in agroforestry is based on interactions between woody and non-woody components notably the woody and herbaceous species that aim to optimize primary production while increasing functional diversity and the resilience of agroforestry systems (AFS). With all their advantages, AFS can be an option for the ecological restoration of one to more than six billion ha of degraded land on earth.

1.3 Introduction

Since the industrial era, land degradation defined as the temporary or permanent decline in the productive capacity of the land, including all its ecosystem services has been advanced as one of the most pressing current global issues (Gerber *et al.*, 2014). The degraded land is estimated about half of world land area and it is still increasing (Gibbs and Salmon, 2015). This brings our global economy and human wellbeing at risk, as land degradation leads to loss of biodiversity, healthy soils and forest cover, water scarcity, food insecurity, and global warming (Ferwerda, 2016).

In general, degraded, damaged or destroyed (3D) lands by mining are characterized by low fertility soil and sometimes high levels of contaminants. Fertilization and soil amendment could be applied to support the plant growth, as it has been shown to improve soil properties and functions relevant to agronomic and environmental performance (Joseph and Lehmann, 2009; Woolf *et al.*, 2010). The use of fertilization and large amounts of organic amendments may help the plants to grow better, but it is very costly and may not be sustainable in the long term. Hence, cost-effective nature-based eco-engineering methods for remediation, reclamation, restoration and rehabilitation (RE4) of mining sites to rebuild the ecosystem services after mining activities cease should be developed (Nadeau *et al.*, 2016).

More recently, a common interest among world leaders and the society at large has emerged in ecological restoration. Indeed, the United Nations (UN) General Assembly declared on 1

March 2020 the UN Decade on Ecosystem Restoration (2021-2030) with the aim as a means to contribute to global efforts to combat climate change and safeguard biodiversity, food security, and water supply. The restoration of over 2 billion ha of degraded ecosystems is of global importance to our economy and in order foster public-private financial partnership to mobilize sufficient resources to restore these lands, Ferwerda (2016) has proposed the "Four Returns" approach. This approach combines the return of Financial Capital with return of Social Capital (jobs), Natural Capital (biodiversity) while connecting the landscape to human resources through returning Inspirational Capital. The ecological restoration industry should be based on four returns sustainable business models in at least a timeline of 20 years (or one generation).

Several definitions have been proposed to "restoration", of which the most commonly used vary from rebuilding the ecosystems using the past reference into the process of assisting the recovery of an ecosystem that degraded, damaged, or destroyed (Clewell *et al.*, 2004). At the same time, the Society for Ecological Restoration (SER) defined restoration as "the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed" (Clewell *et al.*, 2004). Broadly speaking, its means returning a site or system to "pre-disturbance conditions" and implies the follow-up of its recovery process to its future potential to evolve and adapt (Falk *et al.*, 2006). Lima *et al.* (2016) has recently revisited the definitions of commonly used interchangeably or vaguely defined terms remediation, reclamation, restoration and rehabilitation (RE4), ranging from the avoidance of exposure to pollutants (remediation) to the full recovery of the original ecosystem (restoration). Yeldell and Squires (2016) have defined "remediation" as the cleanup of the contaminated area to safe levels by removing or isolating contaminants whereas "restoration" is the process of rebuilding the ecosystem that existed at the mine site (where applicable) before it was disturbed. These authors have defined "reclamation" as the physical stabilization of the terrain (dams, waste rock piles), landscaping, restoring topsoil, and the return of the land to a useful purpose whereas "rehabilitation" as the establishment of a stable and self-sustaining ecosystem but not necessarily the pre-existing one before mining began. In many cases, simple "revegetation" activities which involves using native and nonnative plant species plants to mimic natural ecosystem development over an extended period of time. Whereas "rewilding" refers to the planned reintroduction of a plant or animal species and especially a keystone species into a habitat from which it has disappeared in an effort to increase biodiversity and restore the health of an ecosystem (Gann *et al.* 2019). According to Watson *et al.* (2000), “reforestation” refers to re-establishment of a forest cover in areas where the forest has been cleared in the recent past, usually to repurpose the land for activities like agriculture or mining. While “afforestation” is the process of establishing tree/forest cover in areas that have not been forest in recent history (e.g, deserts, grass savannah). The latter activities can also include various goals, including ecological restoration. Gann *et al.* (2019) have reviewed the most comprehensive international principles and standards for the practice of ecological restoration. In this review, a robust framework for restoration projects is presented to achieve intended goals of restoration, while addressing challenges including effective design and implementation, accounting for complex ecosystem dynamics (especially in the context of climate change) and navigating trade-offs associated with land management priorities and decisions.

This chapter will focus on the natural capital with the application of agroforestry practices on degraded lands as a tool for ecological restoration, rehabilitation and revegetation for the recovery of ecosystem services (provisioning, regulating, supporting, and cultural services) (Atangana *et al.*, 2014; Garrity, 2012; Vieira *et al.*, 2009). This type of agro-succession is a unique practice that has been applied in many areas but less known and rarely published in popular restoration journals (Lamb, 2011; Vieira *et al.*, 2009). Vieira *et al.* (2009) reviewed on the possibility of agro-successional restoration method through agroforestry system with some examples from tropical countries in South America. The forest restoration project in Gunung Kidul - Java, Indonesia, has also been successfully recovered from a highly degraded land through agroforestry by involving the local communities within 30 years (Appanah *et al.*, 2016). The similar agroforestry application under the term “permaculture” has turned severely-degraded dessert land into a fertile edible forest garden in Jordan (Hathaway, 2016). Various agroforestry systems have also been established for long in arid and semi-arid areas as an important source of food and natural resources in Africa (Atangana *et al.*, 2014; Weber and Stoney, 1986) and elsewhere (Atangana *et al.*, 2014; Cooper *et al.*, 1996; Hathaway, 2016).

Intensive management is one of important keys on the success of agroforestry application (Atangana *et al.*, 2014; Cooper *et al.*, 1996). Labor has an important role in intensive management of agroforestry systems for assisted ecological restoration, particularly in industrialized nations where the labor is very expensive (Nair, 2007; Thevathasan *et al.*, 2012). The cost-benefit tradeoffs on the social-economic factors can be an obstacle on its application. But at the same time, these factors can be very dynamic depending on the social and political conditions of the region and the perspective on how we estimate the “benefits”. In a long term, sustainable agroforestry system can be very beneficial when limited land resources and ecosystem services become tangible and valuable (Nair, 2007).

Beyond the challenges on system management, a lot more effort is required on the development of methods for soil and ecosystem remediation as the foundation of the system. Agroforestry has a firm basic science on soil-tree-crop interactions that can be used for exploring the best method for restoring the micro- and macro-ecosystems while accelerating the restoration processes (Lescourret *et al.*, 2015). Agroforestry is a mixture of woody and herbaceous multi-species, which establishes the symbiotic mutualism and provides services required for sustainable ecosystem (Atangana *et al.*, 2014; Ong *et al.*, 2015). This system formed by different functional groups makes it more resilient to disturbances (Choi *et al.*, 2008; Leakey, 2012; Solbrig, 1994). The knowledge on the basic principles and functions of agroforestry is essential for ecological restoration including the multi-species principles, phytoremediation, and facilitation.

1.4 Multi-species principles

Agroforestry systems hold the same principles as a balanced ecosystem with multi-species components, which grow together as one system (Atangana *et al.*, 2014; Ong *et al.*, 2015). A balanced ecosystem can be formed naturally as passive restoration methods. But the natural enrichment process can take decades or even longer depending on their supporting ecosystem (Lamb, 2011). Even though mixed-species plantings were often suggested over monoculture tree plantations to accelerate the restoration processes (Lamb, 2011; Martínez-Garza and Howe, 2003). The current conventional practices with monoculture tree plantations, which rely on plant succession of ecologically well adapted native species, are likely to have evolved some capacity to recover after natural disturbances or stresses (Bremer and Farley, 2010; Gann *et al.*, 2019; Lugo, 1997),

The mixed plantings in conventional restoration was known to be less practical, especially when it is dealing with initial planting strategy, species selection and seeds source availability (Martínez-Garza and Howe, 2003). The known planting strategy is somehow based on monocultural tree plantations with enrichment of mixed species and with no details on how the additional species are supposed to be added. Some studies have suggested to include pioneer and late successional species during the initial planting phase, which is expected to form a good structure of initial stand composition (Corbin and Holl, 2012; Lamb, 2011; Martínez-Garza and Howe, 2003). Others have suggested to incorporate some seed and fruit species to attract the dispersal agents (Holl and Aide, 2011; Martínez-Garza and Howe, 2003). No details are provided on how the enrichment can be done effectively related to plant-plant interactions and how it may affect the stand composition dynamics.

Since the creation of the World Agroforestry Centre (ICRAF) in Nairobi in 1977, research on Agri-silvicultural systems has been inspired by robust science on how to mix the species, namely crops and tree species or broadly speaking herbaceous and wood species termed here as herbosilviculture or herboforestry (Atangana *et al.*, 2014; Dupraz *et al.*, 2019; Nair and Garrity, 2012; Noordwijk *et al.*, 2004; Ong *et al.*, 2015). The research focus is given on the optimum species composition, spatial and vertical arrangements, and also on sequential plantings (Atangana *et al.*, 2014; Ong *et al.*, 2015). Beyond the plant mixture, the systems may also include the animals and their interaction with other components (Atangana *et al.*, 2014). The optimization of the systems is intended to get better growth and productivity while maintaining its sustainability. With robust science principles, the application of agroforestry systems for ecological restoration can improve our understanding on how to accelerate the successional processes on highly degraded lands.

Multispecies systems are crucial for either agroforestry or ecological restoration (Palmer *et al.*, 1997). Plant mixtures have high correlation with ecosystem properties and functional diversity (Choi *et al.*, 2008; Hooper *et al.*, 2005; Schulze and Mooney, 1994). Their impact on primary production is also known to be positive through complementarity effect (niche differentiation of facilitation) or sampling effect (Cardinale *et al.*, 2007; Choi *et al.*, 2008; Hooper *et al.*, 2005; Loreau and Hector, 2001), although it may depend on the observed scales, spatial variability and patterns within the landscape (Swift *et al.*, 2004; van Noordwijk, 2002). Nevertheless, their primary roles on functional ecology for sustainable ecosystem is essential in ecological restoration activities (Hooper *et al.*, 2005; Swift *et al.*, 2004; Vitousek and Hooper, 1994).

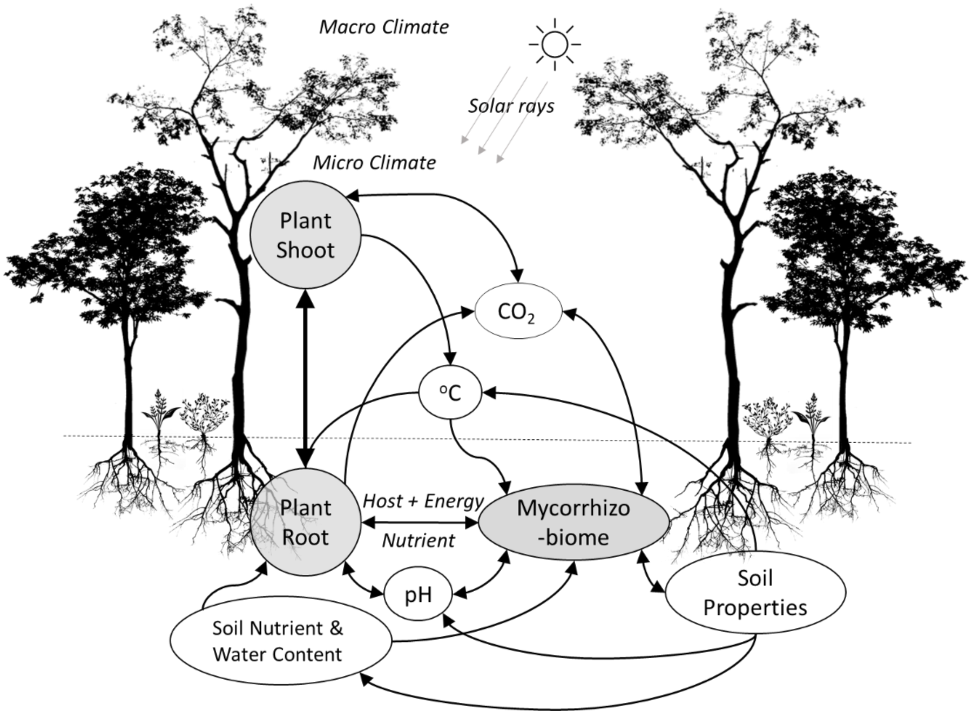

Figure 1.1 Complex ecological interactions between components of phytoremediation multi-species and facilitation principles that should be considered in system intervention for ecosystem restoration.

1.5 Rehabilitative function

The mixed plantations are known on promoting the microbial association in the soil which is important for rehabilitating a degraded soil (Rachid *et al.*, 2013). The plant-microbe associations has a main function as soil ecosystem regulator (Figure 1.1). The plant’s litter influence surface protection, soil fauna activities, and soil microclimates, while microbial processes regulate the nutrient mineralization from mulch and soil organic matter which affect the soil biology, soil chemistry and soil physical properties (Fortin *et al.*, 2015; Smith and Read, 2009; Swift and Anderson, 1994). At the same time, soil ecosystem are formed by various microbial species with specific roles and functions (Paul, 2014), which contribute significantly to shaping the aboveground biodiversity and the functioning of terrestrial ecosystems (Eisenhauer *et al.*, 2010; van der Heijden *et al.*, 1998).

The soil microorganisms have an important role for rehabilitating and remediating the soil (Smith and Read, 2009). Phytobial remediation is a method that uses plants and associated soil microbes to remediate contaminants from soil, sludge, sediments, wastewater or ground water (Ali *et al.*, 2013; Weyens *et al.*, 2009). This method is believed to be environmentally friendly compared to conventional engineering approach (Ali *et al.*, 2013; Doty, 2018a, 2018b). The common phytoremediation method is somehow to only use specific plant species or microbe designated for specific target by monocultural plantations (Ali *et al.*, 2013; Fischerová *et al.*, 2006). The option with mixed species has been suggested, with the species selection intended for phytoremediation (Atangana *et al.*, 2014; Rockwood *et al.*, 2004). Although phytoremediation with mixed species was considered to be impractical and inefficient, the method can give long term guarantees (Rockwood *et al.*, 2004), because the resistance of soil biota and plant growth to disturbance is known to be increasing with plant diversity (Bennett *et al.*, 2020).

Species diversity is known to enhance the ecosystem functioning through interspecific facilitation (Cardinale *et al.*, 2002; Wright *et al.*, 2017). The facilitation factor is very essential on ecosystem restoration, especially on the early stage of succession (Gómez-Aparicio, 2009; Ledo *et al.*, 2014; Padilla and Pugnaire, 2006). The natural regeneration can show a clear effect of facilitation on the coexistence of plant species within the clustered distribution of newly regenerated plants and their nurse plants (Gómez-Aparicio, 2009; Kitzberger *et al.*, 2000; Ledo *et al.*, 2014; Markham *et al.*, 2011). The nurse species provide favorable environment by regulating the microclimate, reduction of water and nutrient stress, and protection from disturbances (Bruno *et al.*, 2003; Callaway, 1995; Callaway and Walker, 1997; Padilla and Pugnaire, 2006). At the same time, there is also trade-off between facilitative effect provided by the nurse species and disadvantages due to competition between neighboring plants (Bruno *et al.*, 2003; Gómez-Aparicio, 2009; Ledo *et al.*, 2014; Ong *et al.*, 2015). The facilitation is often mentioned as a net positive outcome over the negative competitive interaction within the plants (Bruno *et al.*, 2003; Callaway, 1995; Callaway and Walker, 1997; Padilla and Pugnaire, 2006)

The positive effect of density in general was often called as “Allee” effect (Bruno *et al.*, 2003; Callaway and Walker, 1997; Courchamp *et al.*, 2008). The effect indicates a positive density dependence, or the positive correlation between population density and individual fitness and habitat amelioration (Courchamp *et al.*, 2008). The habitat ameliorations include the environmental and microclimate alterations and individual plant interactions by sharing carbon, nutrients, water, defense signals and allelochemicals through mycorrhizal networks (Figure 1.1). Simard *et al.*, (2012) have suggested that these mycorrhizal networks are fundamental agents of complex adaptive ecosystems because they provide avenues for feedbacks and cross-scale interactions that lead to self- organization and emergent properties in ecosystems. To improve the ecological restoration, we suggest to includes the optimization of this “Allee” effect.

1.6 Plantation strategy

Plant spacing can be an important aspect on revegetation activity which may involve exotic or native species, considering the facilitative effect benefit on higher density. Finding the optimum spacing can be important for the successfulness of the revegetation, although facilitative and competitive dynamics may change over time as the forest stand evolve (Holmgren *et al.*, 1997; Kitzberger *et al.*, 2000). Obtaining the best strategy on initial plantation including spacing and species selection can be essential on the restoration processes.

Nucleation method is one of plantation strategies in restoration practices which is inspired by natural colonization processes (Albornoz *et al.*, 2013; Bechara *et al.*, 2016; Boanares and Azevedo, 2014; Corbin *et al.*, 2016; Corbin and Holl, 2012). It involves planting small patches of vegetation (often trees) that attract dispersers and facilitate establishment of new recruits, expanding the forested area over time (Gann *et al.*, 2019). The nucleation approach aims to establish the improved micro environment within the cluster to accelerate the regeneration and ecosystem restoration by the expansion of the cluster (Corbin and Holl, 2012). The method has been shown to improve plant survivability compared to regular plantation (Bertoncello *et al.*, 2016). This could be an indication of Allee effect benefit, as the density was also higher on nucleation method (Bertoncello *et al.*, 2016). An example of natural clustering in harsh condition can be found on tiger bush strips formation in arid Sahel, where a spatial self-organization of vegetation was observed to deal with water limitation (Gilad *et al.*, 2004; Rietkerk and van de Koppel, 2008). Vincenot *et al.* (2016) have developed hybrid models, namely integrated System Dynamics (ISD) and Individual-based (IB) models, to illustrate the importance of individual plant dynamics to explain spatial self-organization of vegetation in arid environments.

The plantation spacing was one of considerations in agroforestry systems and practices (Atangana *et al.*, 2014; Ong *et al.*, 2015). The optimum spacing can be very site specific and depending on the species components of the system. Basic science in plant interaction modelling can be helpful for testing the spacing scenarios on the growth dynamics (Dupraz *et al.*, 2019; Ong *et al.*, 2015; van Noordwijk *et al.*, 2011). The model requires a parametrization adapted to degraded land which might not in the objective of general agroforestry model. The basic research on plant interactions adapted on harsh conditions can help to understand the mechanisms on facilitation and soil rehabilitation, thus accelerate the restoration processes.

1.7 Conclusion

The definition of either ecological restoration, rehabilitation or revegetation has an overlap meaning where each complete each other for ecosystem services recovery. The agroforestry system as a sustainable practice has all the premises for either ecological restoration, rehabilitation, or revegetation of 3D lands. Because of the rehabilitative function over mixed species of agroforestry, the intensification of this practice on 3D lands is an essential eco-engineering tool to rebuild biodiversity, connectivity, ecosystem resilience, ecosystem services; and help mitigate and adapt to the effects of climate change, and ultimately improve human well-being while reducing environmental risks and scarcities. While the cost-benefit tradeoffs on the social-economic factors can be an obstacle on its application, the agroforestry systems may have a high prospect in the long term in the restoration industry. With firm basic science and continuous research on the system adaptation, the agroforestry systems can be a promising solution for anticipated land and food scarcity in the future, during the upcoming UN decade (2021–2030) on Ecosystem Restoration and beyond.

Chapter 2 Tree establishment on post-mining waste soils: species, density and mixture effects

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2.1 Résumé

L'établissement d'arbres dans le cadre de la restauration des terres dégradées après l'exploitation minière est compromis par la faible productivité du sol et les concentrations potentielles élevées de contaminants. La végétation mixte peut entraîner à la fois des effets de compétition et de facilitation, mais un compte rendu mécaniste des interactions et de l'adaptation plante-plante est difficile à réaliser dans les conditions de terrain. Les essais dans des conditions contrôlées de croissance précoce des arbres peuvent distinguer les effets de la densité de plantation et les interactions des espèces.

Un essai en serre a été mis en place dans des conteneurs («mésocosmes») en utilisant des stériles et des résidus fins provenant de mines d'or. Des semis pré-germés (âgés d'une semaine) d'*Alnus viridis* subsp. *crispa*, *Picea glauca*, *Populus tremuloides*, *Salix arbusculoides* ont été plantés dans un dispositif de Nelder, modifié pour les combinaisons d'espèces. Un effet de compétition relative (ECR) a été quantifié comme mesure de la compétitivité pour chaque combinaison d'espèces, calculé comme le rapport des coefficients α dans l'équation de croissance de Holliday. En tant que trait fonctionnel de la plante, la surface foliaire spécifique (SLA) a été mesurée comme indicateur de l'adaptation au stress hydrique de la plante.

Toutes les espèces poussaient mieux en monoculture sur des résidus fins, tandis que seul *P. tremuloides* poussait mieux dans tous les mélanges sur les stériles. Aucun mélange n'apportait d'avantages pour les deux espèces dans des combinaisons appariées. Les effets positifs nets de la densité avec l'augmentation de la SLA au début de la croissance suggèrent une amélioration du microclimat sur les résidus fins.

2.2 Abstract

Tree establishment in restoring degraded post-mining lands is challenged by low soil productivity, a harsh microclimate, and potentially high contaminant levels. Use of mixed vegetation can facilitate microclimate but increase competition for soil resources. A statistical accounting of plant-plant interactions and adaptation to multi-species conditions is hard to achieve in field experiments; trials under controlled conditions can distinguish effects of planting density and species interactions in the early stages of plant establishment.

A greenhouse trial was established in containers (‘mesocosms’) containing waste rock or fine tailings from gold mines. Pre-germinated (one-week-old) seedlings (*Alnus viridis* subsp. *crispa*, *Picea glauca*, *Populus tremuloides*, *Salix arbusculoides*) were planted using a Nelder density gradient design, modified for species combinations. A Relative Competition Effect (RCE) was estimated as a competitiveness index for each species combination, calculated as ratio of α coefficients in the Holliday growth equation. As a plant functional trait, specific leaf area (SLA) was used to indicate plant water stress adaptation.

All species grew better in monoculture on fine tailings, while only P. tremuloides grew better in all mixtures on waste rock. No mixture provided advantages for both species in paired combinations. Net positive effects of density with SLA increment during early growth suggested microclimate improvement on fine tailings.

2.3 Introduction

Challenges for the restoration of degraded post-mining lands include low soil productivity and a high potential for metal toxicity (Markham *et al.*, 2011; Young *et al.*, 2013). Low levels of micro- and macronutrients and a lack of beneficial microbes in mining residues are a challenge for revegetation (Bois *et al.*, 2005). Fertilization can be very costly and natural vegetation can take decades to colonize and remediate post-mining sites in boreal ecosystems (Nadeau *et al.*, 2018a). Cost-effective active restoration is often faced with technical problems such as species selection, plantation method, planting resources etc. (Bechara *et al.*, 2016).

Post-mining sites can be very degraded where the ordinary silvicultural method may not be enough for accelerating plant growth and survival. The physico-chemical and microbiological characteristics of post-mining soil materials prevent desirable rates of plant growth (Nadeau *et al.*, 2018a). For some tree species, mycorrhizal inoculation may improve plant growth and health, together with plant survival, under these stressful conditions (Nadeau *et al.*, 2018a). Another option could be to find an improved method for enhancing the facilitative effect within the plants sharing the space (Bertoncello *et al.*, 2016; Markham *et al.*, 2011; Vieira *et al.*, 2009).

Facilitative effects play an important role in restoration of plant communities in severely disturbed ecosystems with harsh microclimates (Brooker *et al.*, 2008; Markham *et al.*, 2011). The interactions between plant species can be very complex, however, requiring a deep understanding of the trade-offs between positive facilitation and negative competition. These trade-offs need to be managed for successional processes to proceed in severely disturbed ecosystems such as mining sites (Brooker *et al.*, 2008; Markham *et al.*, 2011). Mechanisms may vary, including classical nurse-plant effect interactions, soil nitrogen availability, common mycorrhizal networks, species-specific mutualism interactions, and many others (Brooker *et al.*, 2008; Holmgren *et al.*, 1997). The facilitative effect that emerges from cluster planting might be due, in part, to belowground mycorrhizal activities (Bertoncello *et al.*, 2016; Brooker *et al.*, 2008; Holmgren *et al.*, 1997; Markham *et al.*, 2011; Simard *et al.*, 2012). However, the underlying mechanisms in restoration practices are site-specific and may depend upon species, planting configuration, intervention scenarios, and other factors. Thus, the facilitative effect that is observed in one situation may not be replicable elsewhere, given that it may be influenced by other site-specific factors.

Species interaction mechanisms are important in understanding the interplay between facilitation and competition in plant communities (Holmgren *et al.*, 1997). Different factors may influence species interactions at different densities. Monospecific density experiments are expected to show the “Allee” effect, a benefit of living in groups (Courchamp *et al.*, 1999). The Allee effect is an ecological mechanism of facilitative behaviors in biology and environmental conditioning in plant ecology (Courchamp *et al.*, 1999). The environmental conditioning can be affected by density and may be further enhanced by species mixtures.

Biodiversity is believed to have an important role in ecosystem productivity through complex interactions and facilitative mechanisms which are also known as complementarity and selection effects (Bechara *et al.*, 2016). The complementary effect with niche partitioning in a mixture can lead to overyielding (Van de Peer *et al.*, 2018). Knowledge of the facilitative mechanism of species mixtures is very important for designing an effective plantation method. The experimental separation of density effects and species combinations is required to understand trade-offs and competitive dominance.

Nelder wheel plot experimental designs may be used for efficiently testing the response of plants to planting density. Its purpose serves in comparing multiple tree spacings within a single plot (Nelder, 1962). The plot is circular with the highest density in the center and outward radiating concentric rings, with spokes connecting the center with the furthest ring (Nelder, 1962; Parrott *et al.*, 2012). The original method was developed for density effects in single species, but the design can also be applied to mixed-species plantings with some modifications (Cole and Newton, 1986; Doran *et al.*, 2001; Wurtz, 1995). Its application during initial planting stages of post-mining restoration is expected to indicate the best strategy for demonstrating net positive interactions both between and within species.

In the current study, we evaluated combinations of four woody species that are native to boreal Canada: green alder (*Alnus viridis* (Chaix) DC. ssp. *crispa* (Aiton) Turrill); white spruce (*Picea glauca* (Moench) Voss); trembling aspen (*Populus tremuloides* Michaux); and littletree willow (*Salix arbusculoides* Andersson). These species have different growth forms and successional status. The objective was to determine the effects of mixing species along a density gradient on early growth after tree planting in post-mining waste tailings. We posed the following research questions: 1) Are there any advantages to mixing species over monocultural plantings? 2) Are the effects of mixing species consistent along density gradients? 3) Are there trade-offs between competition and facilitation?

2.4 Material and methods

2.4.1 Tailings

Fine tailings and waste rock came from two gold mining sites, Sigma-Lamaque (now Integra Gold) and Metanor Resources. These were in the Abitibi-Témiscamingue administrative region of Quebec, Canada. Fine-tailing waste materials were generated during gold extraction, while waste rock spoil is unused rock from the mining processes. The fine-tailings are usually deposited in the form of a slurry into tailings ponds that are left to evaporate (Aubertin *et al.*, 1996; Kossoff *et al.*, 2014). This fine-tailing ponds and waste rock dump is subject to remediation and reclamation upon mining closure. Tailings were composed primarily of biotite, an iron-rich mica mineral (Taner *et al.*, 1986). Analyses of element concentrations that were performed by COREM Company-Group Roche Inc. (unpublished data, 2010) included S from 0.48 to 0.51%, Al from 5500 to 6100 mg kg^{-1}, Ca from 21000 to 23000 mg kg^{-1}, Fe from 14000 to 16000 mg kg^{-1}, Mg from 4000 to 4500 mg kg^{-1}, P from 0 to 560 mg kg^{-1}, and K from 86 to 100 mg kg^{-1}, together with other mineral elements that were important for plant growth in low concentrations, such as Zn, Mn, Cu, Mo, and Na. Macronutrients essential for plant growth, but absent from the waste rock and fine tailings included nitrogen (N) and phosphorus (P).

The pH of tailings was alkaline, between 8.55 and 8.68. Arsenic (As) and cyanide concentrations were quite high at 8 to 9 mg kg^{-1} and 3.7 to 6.3 mg kg^{-1} (> standard of 2 mg kg^{-1}), respectively. Fine tailings had very low hydraulic conductivity with 10^{-4} to 10^{-5} cm sec^{-1} and a small grain size of < 74 µm (Aubertin *et al.*, 1996).

In contrast, waste rock exhibited very high hydraulic conductivity, i.e., 10^{-1} to 10^{2} cm s^{-1} and very large particle sizes ranging from sand (625 µm-2 mm) to gravel (4-32 mm) (Kossoff *et al.* 2014). The fine tailings were very homogenous while waste rocks exhibited heterogeneous compositions.

2.4.2 Plant Species

The selected plant species were white spruce (*Picea glauca*), littletree willow (*Salix arbusculoides)*, trembling or quaking aspen (*Populus tremuloides*), and green or mountain alder (*Alnus viridis* subsp. *crispa*). While aspen and spruce are tree species and salix and alder occur as shrubs, any of the four species can be dominant or codominant depending upon the type of habitat Each of these species can be dominant or codominant, depending upon the type of habitats (Barbour and Billings, 2000). The four species are native to North America and are commonly found in the same area. The seed provenances are West Quaco (alder), Apsley (spruce), Hamtown Corner (willow) and Cambridge Narrows (aspen).

White spruce is a large coniferous tree which can be found on a variety of landforms and soil types, with many different plant associates. The tree grows best on well-drained soils and generally occurs in alluvial and riparian zones. Littletree willow is usually found along streams and rivers, or even on the floodplains. This shrub can be useful for stabilizing streambanks and for providing erosion control on disturbed sites; it grows best on w*et al*luvium. Trembling aspen likewise prefers a moist upland wood and can be found on high plateaus, parklands, alluvial terraces, and along watercourses. This species of aspen is the most widely distributed tree in North America, a major cover type across the continent, and common in mixed conifer forests or as a dominant species in many habitats. Green alder is a fast-growing shrub that grows well on poor soils. It may occur as an understory dominant in open conifer or closed deciduous forests. Alder plays a role in reducing soil erosion and helps to stabilize alluvial deposits. In this experiment, we combined all four species at the initial stage of growth and observed the potential benefits of mixing species composition on the restoration processes. The fastest-growing species were aspen and willow, followed by alder. The three species are light-demanding and are considered pioneers, while spruce is a shade-tolerant and slow-growing species and is considered a mid- to late-successional species (Abrahamson, 2015). The species that are listed are tolerant and adapted to poor soils and disturbed sites, and are often used for restoration and rehabilitation projects (Abrahamson, 2015; Esser, 1992; Howard, 1996; Matthews, 1992). Green alder can grow well on poor soils because of its mutual association with nitrogen-fixing actinobacteria (*Frankia* spp.) and mycorrhizas (Roy *et al.*, 2007). This species is typically used for reforestation on infertile soils to increase soil organic matter content.

2.4.3 Experimental design

The experiment uses a small-scale Nelder plot design (Nelder 1962) that was set-up in rectangular containers of 77 x 97 cm and 10 cm depth (with a total volume of 74.69 l) as mesocosm under controlled greenhouse conditions. The plot is shown in Figure 2.1 with outward radiating wheel rings, and the spokes connecting the center with the furthest ring. The wheel radius is calculated as $r_n = r_0 \alpha^n$, with initial radius (r_0) = 5.1 cm and constant increment (α) = 1.3. The constant increment value ($\alpha = 1.3$) was chosen considering the rational generated number of wheels that still fit inside the mesocosm container (8 wheels). While the initial radius ($r_0 = 5.1$) was the minimum radius where 16 seeds can be planted in a circle. Table 1 shows the wheel radius and the growing area with 16 plants per wheel, which was calculated based on the Nelder plot type A1 formula $A_n = r_n^2 \theta (\alpha - \alpha^{-1})/2$, where θ is the angle between the wheel spokes in radians and r_n = radius of the n^{th} arc in cm.

Each Nelder plot contained 16 “spokes” and accommodated 4 replicates of both monoculture treatments and 8 replicates of the two-species mixtures (see Figure 2.1 and Table 2.1). The mixed-species treatments were planted with alternate spokes on half of the circular wheel (8 spokes). *P. tremuloides*, *A. crispa*, *S. arbusculoides*, and *P. glauca* were planted in pair combinations (total of 6 plots). Planting was done in both the fine tailing and waste rock materials so that the total number of experimental plots was 12. The arrangement of a Nelder-plot container is shown in Figure 2.2.

The experimental unit was the individual plant. The monoculture treatment had 2 spokes replication in one plot or container with total of 6 replications in 3 combined plots, while the mixture treatment had 6 spokes replication in one container only. The plants on the border spokes and border wheel were excluded. Figure 2.1 shows the arrangement of individual plants in a full Nelder cycle on each experimental plot. The overall number of experimental units was 6 spokes replication x 6 density levels x 10 species mixtures (6 mixed-species plantings + 4 monocultures) x 2 soil materials for a total of 720 experimental units.

Seeds were first sown and propagated on 1 cm diameter pellets Jiffy-7 Forestry for 10 days (Stuewe & Sons, Inc., Tangent, Oregon 97389 USA). The seedlings were then arranged in Nelder plot design, as shown in Figure 2.1. Plant height was measured 90 days after planting. All plants were measured, except those on the border wheels and border spokes between the treatment blocks, as shown in Figure 2.1 (within the grey area).

The plants for aboveground biomass and leaf area measurement was sampled from two spokes replication on each mixture treatment, and one spoke replication for monoculture treatment on each container. The root biomass was taken for the whole mesocosm container. Leaf area was scanned and measured using WinFOLIA from Regent Instruments Inc (Québec, QC, Canada). The sampled leaf was then dried (at 70°C for 2 days) and weighed to obtain oven-dry mass for Specific Leaf Area (SLA) calculations.

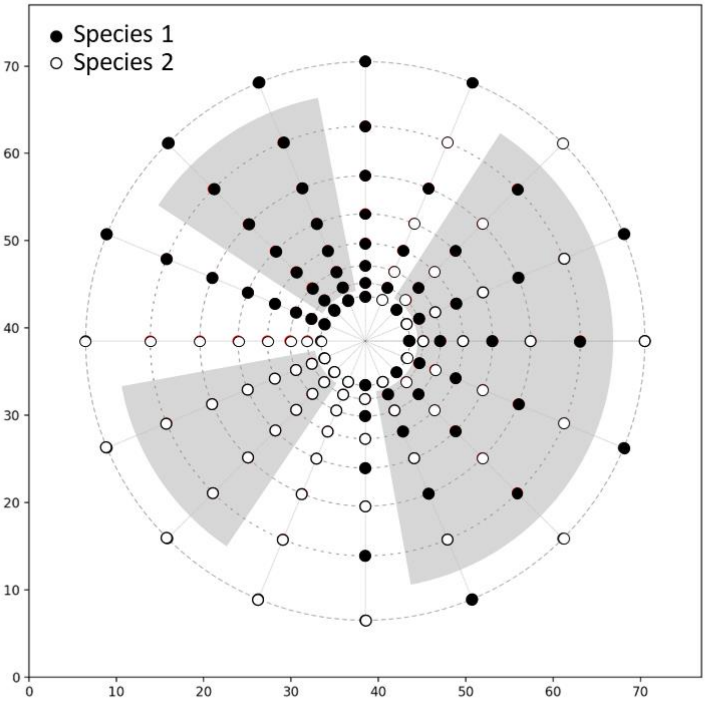

Figure 2.1 The Nelder plot design for tree seedling experiment, the filled and open bullets are seedling positions of two different species, respectively. Number of plants per wheel is 16. The sampled plants are inside the gray area (omitting the plants on the border).

Table 2.1 Wheel radius and spacing area for individual plant in Nelder plot design shown in Figure 2.1.

N	Radius (cm)	Spacing Area (cm^2)
0	5.1	2.7
1	6.6	4.6
2	8.6	7.7
3	11.2	13.0
4	14.5	22.1
5	18.9	37.3
6	24.6	63.0
7	32.0	106.4

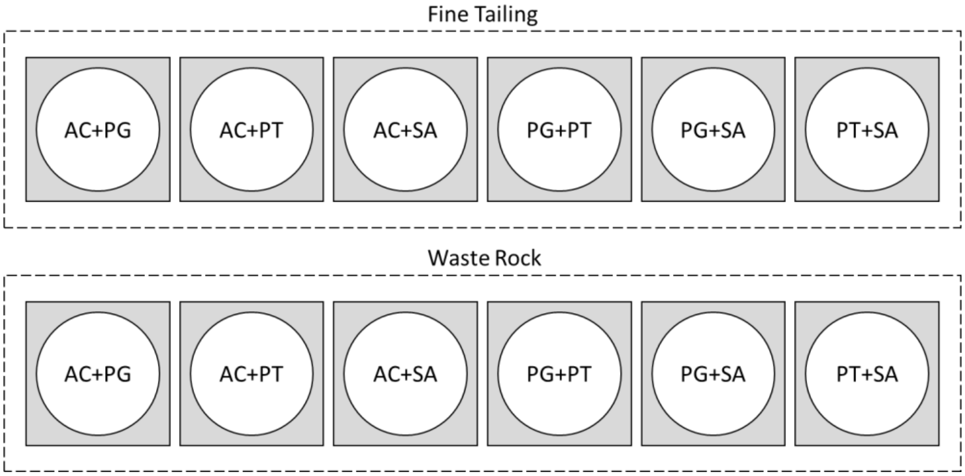

Figure 2.2 The container arrangement for mixed species of A. viridis subsp. crispa (AC), P. glauca (PG), P. tremuloides (PT), S. arbusculoides (SA) in waste rock and fine tailing materials

2.4.4 Growth conditions

Greenhouse temperatures were maintained at 23°C (daytime) and 16°C (night-time), with an average relative humidity of 50%. The greenhouse has additional artificial light using HPS 600 W lamps. The experiment was set up in June 2016 for three months.

Plants were hand-watered daily in the morning (10 mm day^{-1}). Fertilizer was applied weekly using NPK 20-20-20 with the following composition: nitrate nitrogen, 5.9%; ammoniacal nitrogen, 3.9%; urea nitrogen, 10.2%; available phosphoric acid (P_2O_5), 20%; soluble phosphorus (P), 8.7%; soluble potash (K_2O), 20%; soluble potassium (K), 16.6%; iron (Fe), 0.10%; manganese (Mn), 0.05%; zinc (Zn), 0.05%; copper (Cu), 0.05%; boron (B), 0.02%; and molybdenum (Mo), 0.0005%. Fertilizer was mixed in a solution of 50 g per 100l of water (100 ppm) and applied equally across the entire experiment, regardless of substrate.

2.4.5 Data Analysis

The shoot: root biomass ratio of a plant growing in monoculture or mixture reflects the relative ease with which below- and aboveground resources can be obtained. In experiments with *Dactylis glomerate* and *Plantago lanceolate* (Robinson *et al.*, 2010), and *Picea mariana* and *Kalmia angustifolia* (Mallik *et al.*, 2016) no difference was found in shoot: root ratio between the mixture and isolated plants.. Assuming such relation also held in our experiment, we estimated the root biomass of species A as *(AB+AC-BC)/2*, where *AB* is the sum of root biomass of species A and B when combined, *AC* that of A plus C and *BC* that of B plus C, respectively. The formal formula is written as:

$$\bar{y}_a = \frac{y_{ab} + y_{ac} - y_{bc}}{2} \quad (1)$$

where *y* is total root biomass and the letter (*a, b, c*) is the species identifier. The y_{ab} indicates the total root biomass for two species (*a* and *b*) in one container, and $\bar{y}_a$ is the estimated total root biomass for one species (*a*) in the container.

Biomass for individual plants was estimated using the following allometric equation:

$$m = a_i h^{b_i} \quad (2)$$

where *m* is the individual plant biomass, *h* is plant height and a_i and b_i are scaling parameters for species *i*. The scaling parameters (a_i and b_i) were estimated from sampling data for biomass and height using a log-log linear regression model (Harja *et al.*, 2012).

Effects of density on plant height were analyzed using non-linear regression applying the Holliday equation (Holliday, 1960; Willey and Heath, 1969) and modified to incorporate the species mixing treatments is given as follows:

$$w = 1/(\sum \alpha_n i_n + \beta\rho + \gamma\rho^2) \tag{3}$$

where w is individual plant height, ρ is plant density of a specific species, and α, β, and γ are the model parameters. The modified constant α_n is a parameter for each n neighboring treatment and i_n is a dummy variable for identifying the treatment data set (1 = the selected treatment, 0 = everything else). The model allows the α parameter to be fitted among the group of treatments while retaining similar β and γ estimates. The α_n can be interpreted as a measure of plant response on different neighboring species. Therefore, we propose the relative competition effect (RCE) which calculated as follows:

$$RCE = \frac{\alpha_{mono} - \alpha_{mix}}{\alpha_{mix}} \tag{4}$$

where α_{mono} is the model parameter for the monoculture and α_{mix} for the mixed planting. *RCE* is comparable to other measures of competition intensity when β and γ are 0 or without a planting density factor (see Weigelt and Jolliffe 2003). This value is equivalent to the yield relative ratio of the mixed system to the monoculture planting or overyielding (Ong *et al.*, 2015), as shown in the equation below:

$$RCE \cong \frac{w_{mix} - w_{mono}}{w_{mono}} \tag{5}$$

where W_{mono} is the yield from the monoculture system and W_{mix} is the yield from the mixed system. The equation assumes that differences in yield are consistent along the gradient of density following the Holliday equation.

The Holliday equation is suggested for Nelder plot data analysis and general yield density study because the estimators of its parameters are effectively unbiased and normally distributed (Gillis and Ratkowsky, 1978). The original equation of Holliday is as follow:

$$w = 1/(\alpha + \beta\rho + \gamma\rho^2) \tag{6}$$

The biological interpretation of α is a measure of species genetic potential and β is a measure of environment potential, while γ shows curvature of the responses (Gillis and Ratkowsky, 1978; Willey and Heath, 1969).

All analyses were conducted using R software version 3.5.1 (R Core Team, 2018). We used the standard library for linear and nonlinear models and the lmerTest package library for mixed models.

2.5 Results

The allometric regression fit shown a significant parameter result for all species. The allometric regression parameters and adjusted R-squared is shown on Table 2.2. The allometric equation with plant height can predict about 70-75% variation of aboveground plant biomass according to the R-squared result. Since the equation is fitted with log-log linear model, the presented *a* parameter was back-transformed from the original output (*log(a)*) and corrected with mean squared error of the regression. This allometric parameter is later used for estimating the total above ground biomass for the whole plants.

Table 2.2 Regression fit parameters for biomass-height allomeric.

Species	*a*	*b*	R^2
A. viridis subsp. *crispa*	0.0206***	1.94***	0.687
P. glauca	0.0038***	2.28***	0.746
P. tremuloides	0.0044***	1.73***	0.733
S. arbusculoides	0.0184***	1.32***	0.760

Note: *Significant codes: P < 0.001 '***'*

Total biomass growth on waste rock was higher than on fine tailings (Figure 2.3 and Figure 2.4). The fast-growing *S. arbusculoides* and *P. tremuloides* have the highest total biomass on both materials (Figure 2.3). *S. arbusculoides* and *A. crispa* have higher aboveground biomass on waste rock, while *P. tremuloides* and *P. glauca* had higher aboveground biomass in fine tailings.

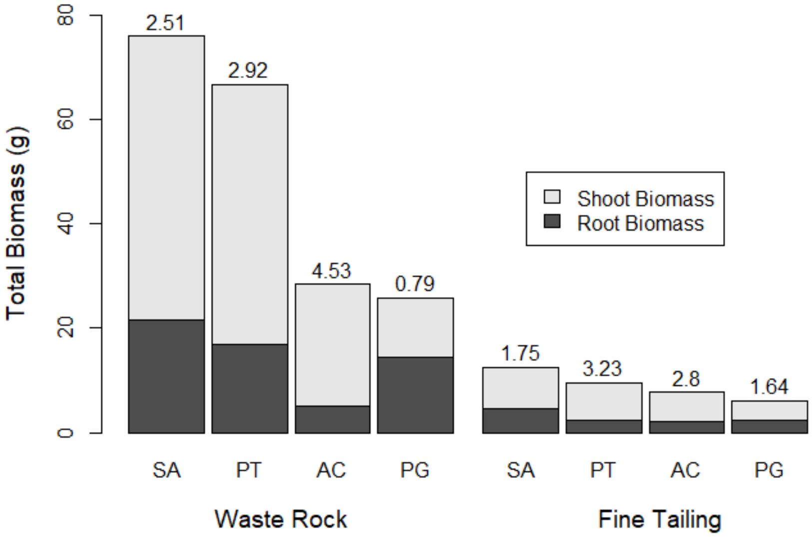

Figure 2.3 Estimated total root and shoot biomass for A. viridis subsp. crispa (AC), P. glauca (PG), P. tremuloides (PT), S. arbusculoides (SA) in waste rock and fine tailings. The value above the bars is shoot: root ratio.

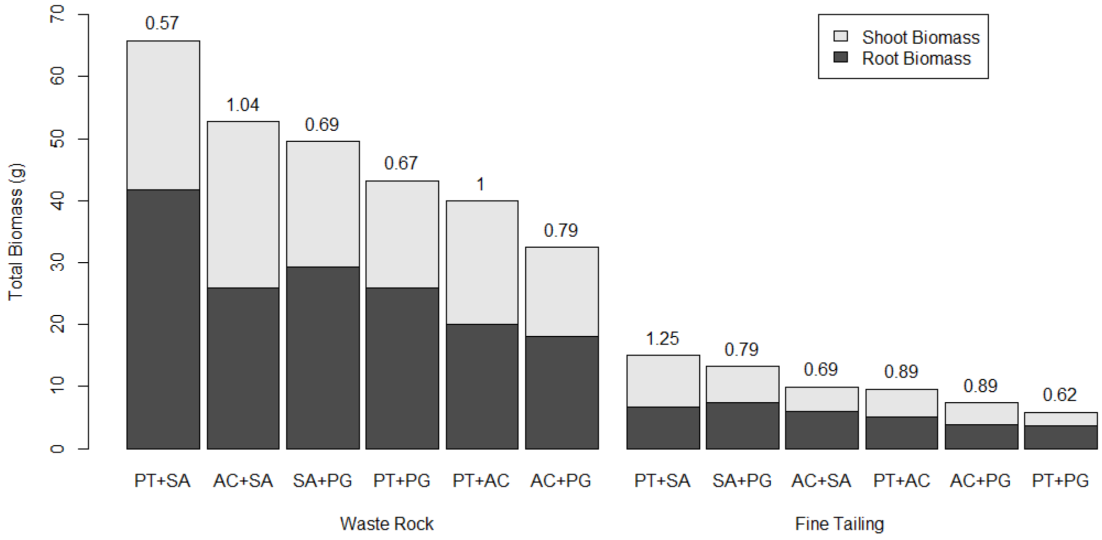

Figure 2.4 Total shoot and root biomasses in each mesocosm container of Nelder plot experiment for combinations of A. viridis subsp. crispa (AC), P. glauca (PG), P. tremuloides (PT), S. arbusculoides (SA) in waste rock and fine tailings. The value above the bars is shoot:root ratio

Interactions between species also influenced early biomass growth. Combining both fast-growing species (*P. tremuloides* and *S. arbusculoides*) led to the greatest biomass growth on both substrates (Figure 2.3), but of all species tested, only *P. tremuloides* increased biomass growth in mixtures. *S. arbusculoides* and *A. crispa* have higher aboveground biomass on waste rock, while *P. tremuloides* and *P. glauca* had higher aboveground biomass in fine tailings. Yet, the ordering from the highest to the lowest biomass apparently differs between waste rock and fine tailings, thereby showing differing performance of species between the two materials. Differences between materials are also reflected in shoot: root ratios. But since species root biomass was estimated from total root biomass in a Nelder container with mixture of species, we can not conclude its correlation with the material factor.

Regression parameters of Holliday equation is shown on Table 2.3 for plants that are grown in waste rock, and Table 2.4 for fine tailing. The curve plot is shown in Figure 2.5 and Figure 2.6 subsequently. The α parameter is significant which shows a constant deviance in yields between all the treatments for all species in waste rock and fine tailing. The significance of β and γ parameter was vary between species, showing a high variation in magnitude or slope and the curvature on the correlation of density and plant height. The highest β is shown for *A. crispa* on waste rock material, and the smallest on *S. arbusculoides*. On the same time, *S. arbusculoides* shown to have high degree variation of data with its high RSE and non-significant β and γ. The non-significant β and γ is also shown for *A. crispa* and *P. glauca* on fine tailing. An interesting result shown for *P. glauca* and *S. arbusculoides* on fine tailing with a contrasting difference of β and γ. The β and γ have signs opposite (-/+) to the normally expected values.

Table 2.3 Regression fit parameters of Holliday equation for the plants grown in waste rock.

Species	α_{AC}	α_{PG}	α_{PT}	α_{SA}	β	γ	RSE
A. viridis subsp. *crispa*	0.28***	0.29***	0.30***	0.22***	0.16*	-0.05	1.15
P. glauca	0.21***	0.20***	0.27***	0.21***	0.11***	-0.03**	0.73
P. tremuloides	0.03***	0.03***	0.05***	0.03***	0.13***	-0.04***	2.80
S. arbusculoides	0.11***	0.07***	0.12***	0.11***	0.04	-0.01	3.62

Note*: The parameter index for α_n was replaced by its corresponding neighbouring species identifier. RSE is the residual standard error of the model estimation. Significant codes: P < 0.001 '***', P < 0.01 '**', P < 0.05 '*'*

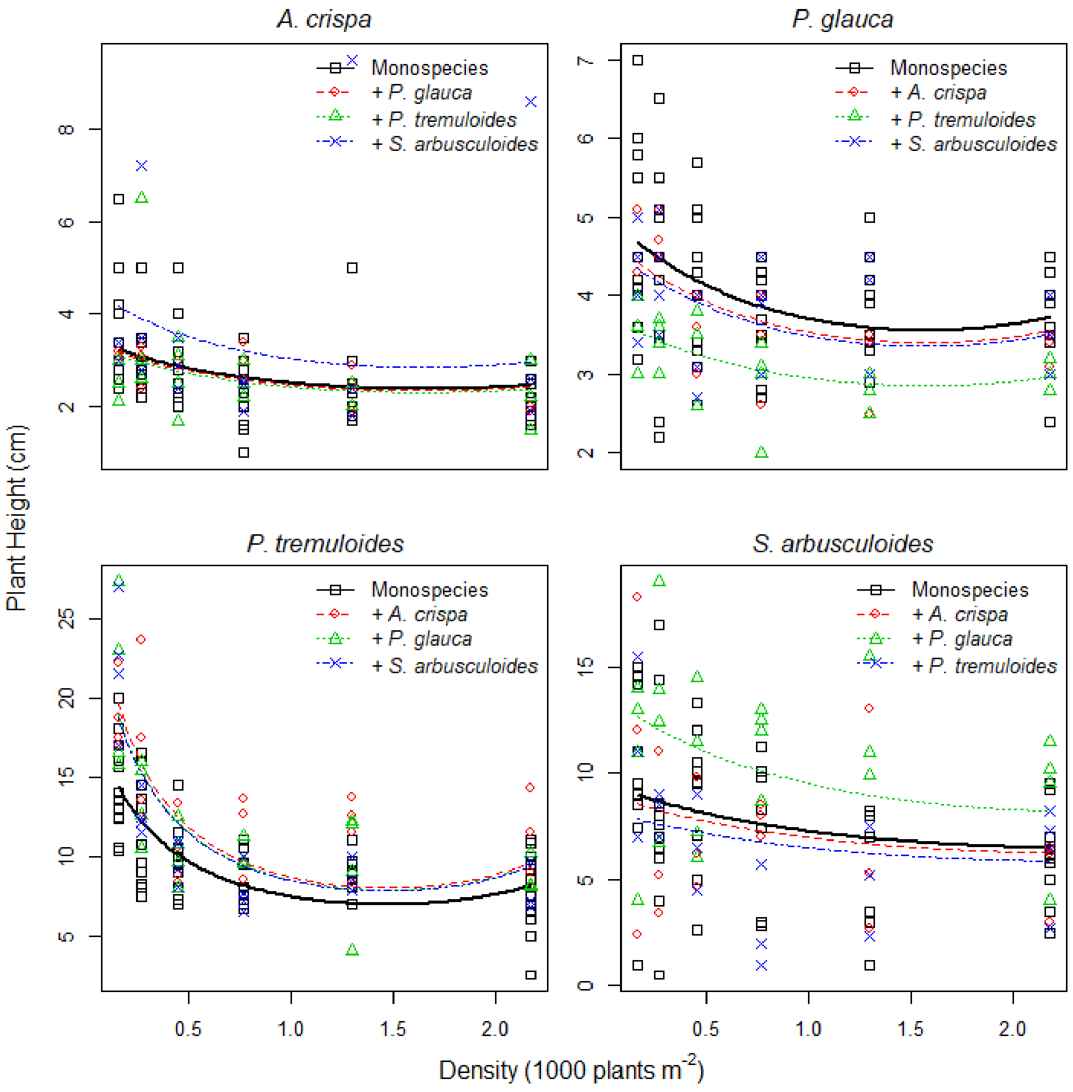

Figure 2.5 The regression curves of Holliday equation on plant height with gradual density and combination of neighbors (showed by different legends) growing in waste rock.

Table 2.4 Regression parameters of Holliday equation for the plant grown in fine tailing.

Species	α_{AC}	α_{PG}	α_{PT}	α_{SA}	β	γ	RSE
A. viridis subsp. *crispa*	0.55***	0.64***	0.62***	0.80***	0.14	-0.04	0.45
P. glauca	0.44***	0.43***	0.45***	0.44***	-0.05	0.01	0.44
P. tremuloides	0.10***	0.30***	0.09***	0.08***	0.28***	-0.09**	2.42
S. arbusculoides	0.65***	0.52***	0.88***	0.55***	-0.33**	0.10*	1.46

***Note**: The parameter index for α_n is replaced by its corresponding neighbouring species identifier.*

*Significant codes: $P < 0.001$ '***', $P < 0.01$ '**', $P < 0.05$ '*'.*

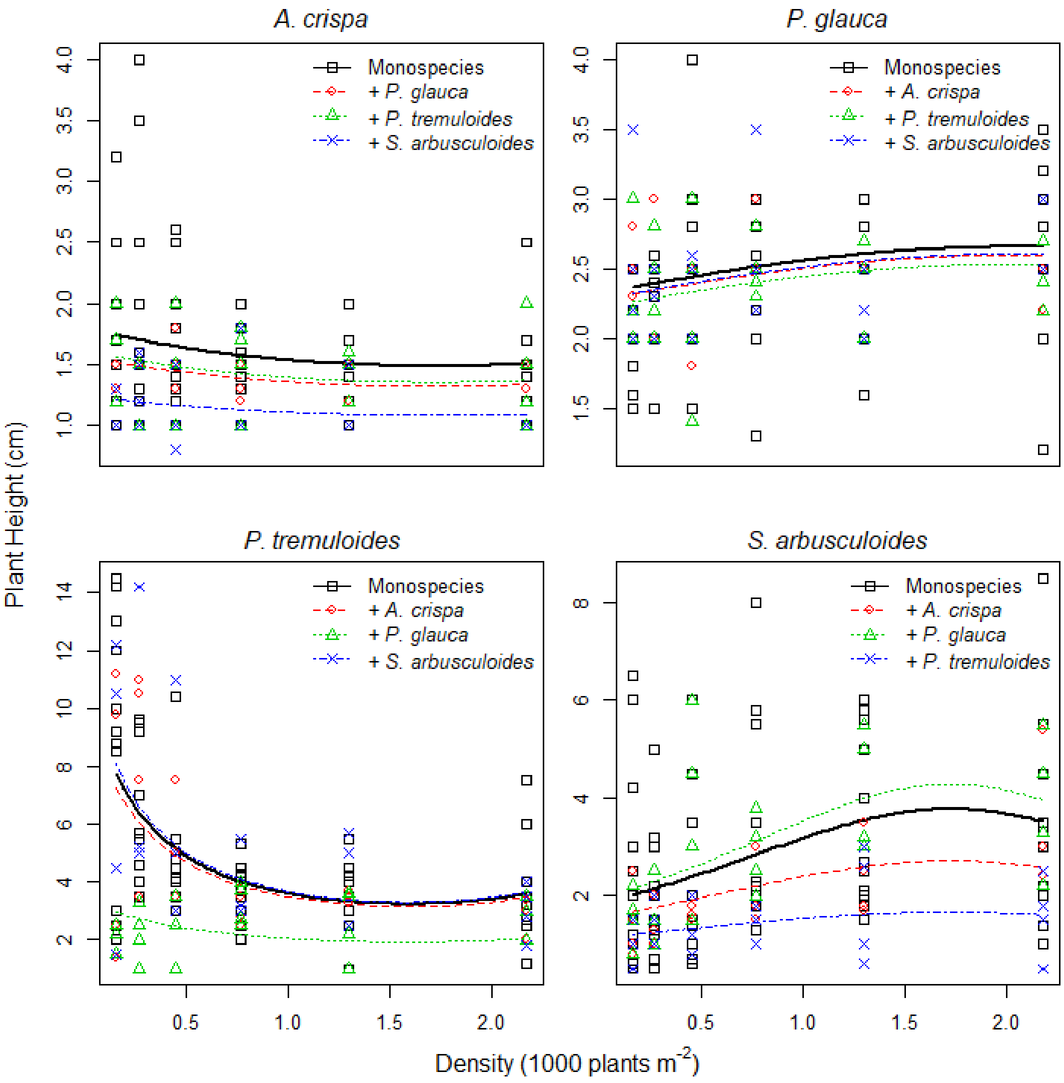

Figure 2.6 Regression curves of Holliday equation on plant height growth with gradual density and combination of neighbors (showed by different legends) in fine tailing.

The Relative Competition Effect (RCE) calculations from the estimated regression parameters are shown in Table 2.5 for waste rock and in

Species	Neighbours			
	A. viridis subsp. *crispa*	*P. glauca*	*P. tremuloides*	*S. arbusculoides*
A. viridis subsp. *crispa*	0.00	-0.02	-0.05	0.32
P. glauca	-0.06	0.00	-0.26	-0.08

P. tremuloides	0.60	0.49	0.00	0.47
S. arbusculoides	-0.05	0.45	-0.13	0.00

Table 2.6 for fine tailings. The RCE has shown that *P. tremuloides* is the most competitive species in waste rock (Table 2.5). It had better yield for about 60% (RCE = 0.6) more when it is planted in mixtures with *A. crispa*, 49% (RCE = 0.49) with *P. glauca* and 47% (RCE = 0.47) with *S. arbusculoides*. Which on the same time its neighbors' losses the yields for about 26% (RCE = -0.26), 13% (RCE = -0.13) and 5% (RCE = -0.05) accordingly. *P. glauca*, in opposite, is always supressed in mixture and losses the yield for about 6% (RCE = -0.06) when it is planted with *A. crispa*, 26% (RCE = -0.26) with *P. tremuloides* and 8% (RCE = -0.08) with *S. arbusculoides*.

Table 2.5 Relative competition effect (RCE) for Nelder experiment applied in waste rock.

Species	Neighbours			
	A. viridis subsp. *crispa*	*P. glauca*	*P. tremuloides*	*S. arbusculoides*
A. viridis subsp. *crispa*	0.00	-0.02	-0.05	0.32
P. glauca	-0.06	0.00	-0.26	-0.08
P. tremuloides	0.60	0.49	0.00	0.47
S. arbusculoides	-0.05	0.45	-0.13	0.00

Table 2.6 Relative competition effect (RCE) for Nelder experiment applied in fine tailing.

Species	Neighbours			
	A. viridis subsp. *crispa*	*P. glauca*	*P. tremuloides*	*S. arbusculoides*
A. viridis subsp. *crispa*	0.00	-0.13	-0.11	-0.31
P. glauca	-0.02	0.00	-0.04	-0.02

P. tremuloides	-0.09	-0.71	0.00	0.06
S. arbusculoides	-0.16	0.06	-0.38	0.00

Fast-growing species seem to dominate in competitions with slow-growing species in waste rock. Except for *A. crispa* and *S. arbusculoides*, where the slow-growing species (*A. crispa*) is shown to be more competitive than the fast-growing species (*S. arbusculoides*). *A. crispa* had 32% (RCE = 0.32) greater yield when mixed with *S. arbusculoides*, while *S. arbusculoides* lost 5% (RCE = 0.05). In contrast, *A. crispa* and *P. glauca* have reduced yields in mixtures for both species, compared to their monocultures (Table 2.5).

Almost all the species were supressed when grown in mixtures on fine tailings as shown on

Table *2.6*. *P. tremuloides* had the lowest losses when it was mixed with *P. glauca* (RCE = -0.71), which is opposite to the response that is observed when both species are planted in waste rock. The only species that showed benefits was *P. tremuloides* when it was mixed with *S. arbusculoides* (RCE = 0.06), and *S. arbusculoides* when it was mixed with *P. glauca* (RCE = 0.06). In general, we could not find any mixture that gave advantages for both species in a paired combination on any of the soil materials.

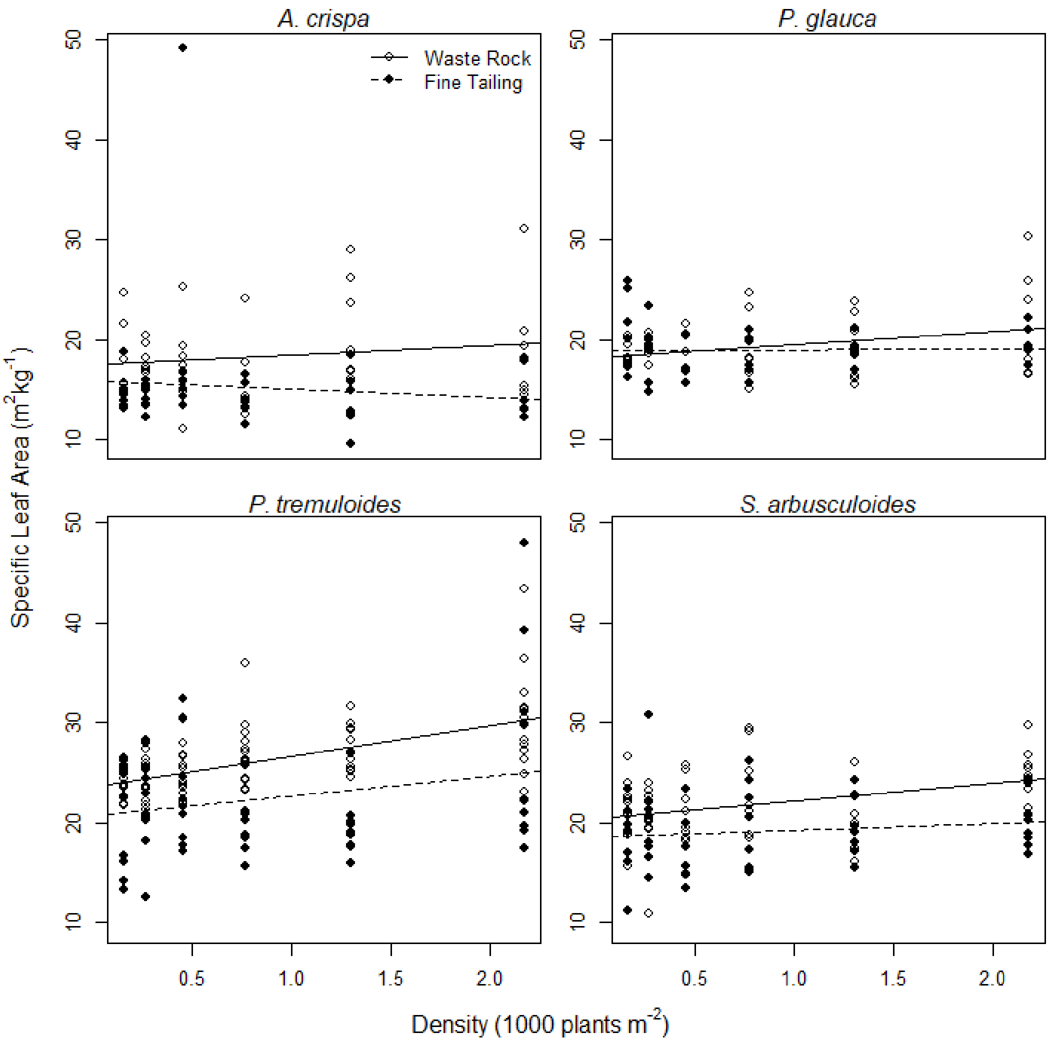

Figure 2.7 Specific Leaf Area (SLA) of individual plants along the density gradient and in waste rock and fine tailings.

Table 2.7 Type III Analysis of Variance Table with Satterthwaite's method for the effect of species, density and material on Specific Leaf Area

Source	DF	F value	Pr(>F)	
Density	1	15.326	0.000	***
Species	3	7.0038	0.000	***
Materials	1	4.115	0.043	*
Density:Species	3	3.4499	0.017	*
Density:Materials	1	5.1531	0.024	*

Species:Materials	3	1.1984	0.310
Density:Species:Materials	3	0.113	0.953

Significant codes: P < 0.001 ‘***’, P < 0.01 ‘**’, P < 0.05 ‘*’

The results from the specific leaf area (SLA) measurement and the statistical analysis of variance are shown in Figure 2.7 and in Table 2.7, respectively. Density gradient and species had significant effects on SLA ($P < 0.01$?). The interaction of density gradient and species also had significant effects on SLA ($P < 0.05$), while the addition of soil material factors to this interaction was not significant. The soil material itself had a significant effect on SLA ($P < 0.05$). The SLA on fine tailings was lower than that on waste rock soil material for most of the species (Figure 2.7) . SLA and density were positively correlated for most species combinations except for *A. crispa* and *P. glauca* in fine tailings (Figure 2.7).

2.6 Discussion

Overall plant growth in waste rock was better than in fine tailings, which could be due to the physical characteristics of the material. The fine grain size of mine tailings results in very high water retention and very low hydraulic conductivity (Aubertin *et al.*, 1996), which are unfavourable conditions for plant species, generally. The measured fine tailings moisture content in the field site is about 20-30% at depth 15 cm. This quantity of water corresponds to the permanent wilting point for fine-grained material, such as clay or silty-clay, which have similar grain sizes in mine tailings. In contrast, waste rock has very low water retention and very high hydraulic conductivity, which is also an unfavourable condition for plant growth, but the waste rock offered a better environment than did fine tailings when it was subject to daily watering and weekly fertilization. In addition, some contaminants in fine tailings could limit growth because of their phytotoxicity.

Indeed, fine tailings contained contaminants such as aluminum, iron, magnesium, arsenic and cyanide. Field observations on similar tailings in the region show natural vegetation, which was believed to be associated with plant adaptations to on-site conditions (Abdul-Wahab and Marikar, 2012). Despite phytotoxicity, plant tissues have shown translocation of metals from roots to the upper parts of the plants (Abdul-Wahab and Marikar, 2012). Some plants are known to have adaptability and resistance to soil contaminants, either through stress avoidance or stress tolerance (Punz and Sieghardt, 1993).

Indeed, species of aspen (*Populus* spp.) and willow (*Salix* spp.) are known heavy metal accumulators in phytoremediation practices (Fischerová *et al.*, 2006; Hassinen *et al.*, 2009; Mehes-Smith and Nkongolo, 2015; Salam *et al.*, 2016). Observations on aspen on metal-contaminated sites showed a high level of mixoploidy in the plants. These mitotic abnormalities have been shown to exert no adverse effects on plant growth and survival (Hassinen *et al.*, 2009; Mehes-Smith and Nkongolo, 2015). In some phytoremediation experiments, the growth of willow was also demonstrated to be unaffected by heavy metal contaminants (Fischerová *et al.*, 2006; Salam *et al.*, 2016). *A. crispa*'s status is less known as a heavy metal accumulator, but it has resistance to organic and inorganic soil contaminants. It is often used for phytostabilization and as nurse species in restoration practices (Lalancette *et al.*, 2019; Roy *et al.*, 2007). *P. glauca*, in contrast, is quite sensitive and less tolerant of heavy metal contamination. An experiment with *P. glauca* on heavy metal-contaminated soil showed about a 20 to 25% reduction in growth (Dixon and Buschena, 1988; Nosko *et al.*, 1988). Thus, growth reduction of *P. glauca* on fine tailings, as shown in our experiment, could be affected by a combination of water stress and phytotoxicity.

Another plant trait characteristic that may differentiate species metabolic strategies in a stressed environment is the shoot: root ratio. The shoot: root ratios for *P. tremuloides* were respectively 2.95 and 3.23 in waste rock and fine tailings, which were still in the range 2-4 for normal conditions (Peng and Dang, 2003). *S. arbusculoides* had respective shoot: root ratios of 2.51 and 1.75 in waste rock and fine tailings, which were lower than the normal 2.5-5 for *S. arbusculoides* (Dušek and Květ, 2006). *A. crispa* showed respective shoot: root ratios of 4.53 and 2.80 in waste rock and fine tailings, which were far higher than the normal ratio 1 – 1.5 for *A. crispa* (Lorenc-Plucińska *et al.*, 2013). The shoot: root ratios for *P. glauca* were respectively 0.79 and 1.64 in waste rock and fine tailings, which were slightly lower than the normal 2 – 4 (Peng and Dang, 2003). Thus, shoot: root ratios were either higher or lower than normal range, similarly for waste rock and fine tailings. This could be due to the similar treatments of daily watering and weekly fertilization on both waste rock and fine tailings.

However, there were some differences in shoot: root ratios within species groups in response to different tailings. The differences were also shown in the mixtures of species combinations, possibly due to different metabolic strategies of each species in tailing materials and interactions with neighbours. While the species root biomass was estimated from a mixture plantation with additive assumption, the conclusion may need to be explored further with more precise experiments.

2.6.1 Density gradient effect

The effect of the gradient of density on mixtures of species was consistent in all treatments. The regression fit of Holliday equation was very significant for all groups. The equation intercept (α) is also significant for all species combinations, whether on waste rock or on fine tailings. Significant alpha parameters for all species mixtures on both substrates suggest the neighboring species influenced biomass growth across all conditions.

A. crispa was found to have the biggest β on waste rock material, which also means that it has high sensitivity to planting density. While *S. arbusculoides* had the smallest β and also small γ value near to zero, which also means that it has more linearity on its correlation (Table 2.3). With high Residual Standard Error (RSE) value and insignificant β and γ, *S. arbusculoides* shown to have more variation error on its correlation data.

A difference was shown on β and γ values for *P. glauca* and *S. arbusculoides* in fine tailings (Table 2.3 vs Table 2.4), with opposite signs (-/+) meaning the greater growth yield, the higher the planting density (see Figure 2.6). This was unusual, as we expected higher competition with increased density. This positive effect of density can be explained by the Allee effect (Courchamp *et al.*, 1999), resulting in a net positive effect between facilitation and competition.

Another possibility of greater plant growth in denser plantings is the “elongation” effect because of competition for light. Since the plants were very small, we were assuming that the light resources might not be limiting in this 3-month experiment. Analysis of the allometric correlation between biomass and height did not reveal a significant effect of density for any species (data not shown).

Observations on the plant functional trait SLA as an indicator of plant water stress adaptation provided interesting information. Most species are known to decrease their SLA as water stress increases (Liu *et al.*, 2016; Marron *et al.*, 2003). The deciduous *P. tremuloides, S. arbusculoides* and *A. crispa* had similar SLA adaptations to water stress (Hennessey *et al.*, 1985; Splunder *et al.*, 1996). Here, we found that SLA was lower in fine tailings compared to that in waste rock for most species, except *P. glauca* (Figure 2.7). Considering the general SLA adaptation for water stress as mentioned above, plants on fine tailings were shown to have higher water stress compared with those on waste rock. *P. glauca* exhibited greater SLA under higher water stress. This response was not surprising given that this conifer was the most drought-tolerant species among those studied in our experiment, and as has been reported elsewhere (Abrahamson, 2015; Moran *et al.*, 2017; Van den Driessche, 1991).

At the same time, we also found greater SLA as the planting density increased, especially for *P. tremuloides* and *S. arbusculoides* (Figure 2.7). Assuming SLA adaptation, this could be an indication of contrasting water stress between lower and higher planting densities. While higher stress can be caused by increased competition at higher density, SLA showed a decrease in water stress. This was consistent with observations on plant height that have been discussed above, where we showed a reversed regression curve on the gradient of density analysis. Thus, SLA could be considered as an indicator of plant facilitation on density gradient experiment.

Plant facilitation does seem to emerge in *A. crispa* on the fine tailings. The SLA tends to have negative correlations with the gradient of density. While *A. crispa* is known as high moisture-demanding species (Matthews, 1992), the competition at increased density seems to be higher than the facilitation effect on water. Thus, *A. crispa* may still exhibit higher water stress at increased planting densities.

The significant effect of the density gradient on SLA adaptation may indicate a facilitative effect on soil moisture. One possible factor relating to soil moisture conservation under higher plant densities is microclimate improvement, soil amelioration with higher root densities, or its combination. The effect of density on microclimate improvement is a known, important aspect of restoration practices (Bechara *et al.*, 2016; Courchamp *et al.*, 1999). We posit that the positive density effect that was observed in our experiment is correlated with microclimate improvement. Further exploration into the details of microclimate factors such as temperature, moisture and other functional traits might be required to confirm our hypothesis.

2.6.2 Mixture effect

The Relative Competition Effect (RCE) measured relative advantages between species mixtures. We found that a mixture does not have advantages for combined pairs of species. Most of the species grow better in monoculture, except for *P. tremuloides* in waste rock. *P. tremuloides* is shown as the most competitive species and *P. glauca* is the most suppressed species. Fast-growing species seem to be the most competitive over slower-growing species in waste rock. Yet, this is not necessarily true for *S. arbusculoides* and *A. crispa*. *S. arbusculoides* has faster growth than *A. crispa* but was suppressed when they were both placed into mixture. Green *A. crispa* also showed lower yield when it was mixed with the slower-growing species *P. glauca* (although *P. glauca* also had lower yield when it was planted alone in monoculture). Belowground competition seems to be the most significant effect in our experiment, since the plants were very small, and light was not limiting at early growth stages. Another possible factor is allelopathy, but this is unlikely given that no reports of allelopathic effects have been found in the literature for any of the species under study.

The monoculture planting performance was better in fine tailings, except when *P. tremuloides* was mixed with *S. arbusculoides,* and when *S. arbusculoides* was mixed with *P. glauca*. *P. tremuloides* was no longer dominant in fine tailings when it was planted in mixtures. At the same time, *P. glauca* performance was better in monoculture both in fine tailings and in waste rock. This experiment was an early-stage evaluation and might not depict the whole suite of interactions that are likely to occur between species at later stages in the greenhouse or in the field. The outcome could be different as the plants grew larger and started to shade one another, exerting both below- and aboveground interaction effects.

2.7 Conclusion

Plant growth was five times greater on waste rock than in fine tailings with similar treatments. Species shoot: root ratios varied from the normal ranges of typical species but shown to have similar deviation between waste rock and fine tailing. Further experiment with more precise root measurement may be required to get the real effect of the treatments.

Positive effects of density on early growth suggested that microclimate improvement played a role in accelerating the growth of the plants. Specific leaf area (SLA), as a plant trait proxy for water stress adaptation, was greatly affected by the material and gradient density. The positive correlation of SLA with density could be an indication of facilitative effects on water stress. This finding could be further investigated for quantifying facilitative and competitive effects on planting density experiments.

We found that mixtures do not offer advantages for both species in paired combinations. Most species grew better in monoculture, but some species grew better in mixtures. The fast-growing *P. tremuloides* was dominant and better in mixtures on waste rock material. Other than this response, we could not find a general correlation between species-specific traits with their adaptations to different mixtures.

The relative competition effect (RCE) quantified the advantages and disadvantages of the mixture over monoculture with proportional assumptions regarding the density gradient. Given that the experiment was of limited duration, the result may not depict a complete picture of plant-plant interactions. We suggest further experiments with longer timelines to better explore the facilitative indicators, together with competition effects, to improve the prediction method in successional dynamics modelling.

Chapter 3 Post-mining restoration strategy: the effect of biochar amendment, microbiome inoculation, crop mixture and planting density on initial plant growth

3.1 Résumé

La restauration écologique avec des espèces multiples et une approche multifonctionnelle peuvent accélérer le rétablissement de nombreux services écosystémiques. Les défis des terres dégradées, endommagées ou détruites (3D) après l'exploitation minière sont la faible productivité du sol et le potentiel élevé des contaminants. Ici, nous avons évalué l'approche multi spécifique et multifonctionnelle de la stratégie de restauration à travers un mélange d'espèces ligneuses et herbacées, d'amendements de micro-symbiotes et de biochar, ainsi que l'espacement des plantations.

Les expériences ont été menées à l'aide d'essais en serre et sur le terrain. Les expériences en mésocosme en serre ont consisté à planter un mélange d'espèces d'arbres (*Alnus viridis* (Chaix) DC. ssp. crispa (Aiton) Turrill, *Picea glauca* (Moench) Voss, *Populus tremuloides* Michx. et *Salix arbusculoides* Andersson) et des espèces herbacées (*Avena sativa* L., *Festuca rubra* L. *et Trifolium repens* L.) sur deux types de rejets miniers aurifères (résidus miniers fins et stériles). Les essais sur le terrain ont été établis sur des sites post-miniers dans le nord-ouest du Québec, au Canada, sur des résidus fins et des stériles, en utilisant un mélange similaire d'arbres et d'espèces herbacées que pour l'expérience en serre. L'amendement du biochar et l'inoculation microbienne ont été appliqués aux essais en serre et au champ. La performance de croissance des plantes a été évaluée après trois mois pour les essais en serre et deux sessions de croissance pour les essais en champ.

Nous avons trouvé des effets positifs et négatifs sur l'espacement des plantations, l'amendement du biochar et l'inoculation en fonction de leurs états d'interaction. L'effet positif net a été démontré en combinant une densité de plantation élevée, du biochar et des facteurs d'inoculation sur *A. viridis* ssp. crispa. Dans l'ensemble, la densité de plantation s'est révélée être le facteur le plus important pour générer l'effet positif net. Nous suggérons que le mécanisme était corrélé à l'amélioration du microclimat par la conservation de l'eau des plantes du sol et l'amélioration de l'activité microbienne par rapport à la modification de la

température du sol. Par conséquent, mettre l'accent sur l'amélioration du microclimat, ainsi que sur d'autres facteurs combinés, y compris l'inoculation microbienne et l'amendement du biochar, est très important pour accélérer les processus de restauration.

3.2 Abstract

Ecological restoration with multispecies and multifunctional approach can accelerate the re-establishment of numerous ecosystem services. The challenges with post-mining degraded, damaged, or destroyed (3D) land are the low productivity of soil and high potential contaminants. Herein we evaluated the multispecies and multifunctional approach on 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. The mesocosm experiments in greenhouse consisted of planting 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 waste gold mine materials (fine tailing and waste rock). The field trials were established on gold post-mining sites in Northwest Quebec, Canada, on fine tailing and waste rock, using a similar mixture of tree and herbaceous species as for the greenhouse experiment. The biochar amendment and microbial inoculation were applied on both greenhouse and field trials. The plant growth performance was assessed after three months for the greenhouse trials and two growing sessions for the field trials.

We found 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, the plantation density was shown as the most important factor in generating the net positive effect. We suggest that the mechanism was correlated with the microclimate improvement through soil plant water conservation and microbial activity enhancement over soil temperature modification. Hence, putting emphasis on microclimate improvement, along

with other combined factors including microbial inoculation and biochar amendment is very important for accelerating the restoration processes.

3.3 Introduction

Since the industrial era, land degradation, damage, and destruction (3D) (hereafter, collectively referred to as degradation), has become one of the most significant environmental problems globally. These problems has diminish the biodiversity, functioning, and resilience of ecosystems, which in turn negatively affects the resilience and sustainability of social–ecological systems (Gann *et al.*, 2019). The tradeoffs between economic benefits and the loss of ecosystem functions seem to be not sustainable in terms of natural resources. Ecological restoration paradigm which focuses on enhancing ecosystem services and increasing resilience is believed to be the best environmentally sustainable practice (Choi *et al.*, 2008). Accelerating land restoration with similar principles could be beneficial considering the expansion in demand for land and food (Vieira *et al.*, 2009).

Multi-species mixtures of trees and crops in agroforestry brings about a unique set of ecological interactions which can be positive, neutral or negative among the different species (Jose *et al.* 2009; Atangana *et al.* 2014; Ong *et al.* 2015). If the agroforestry system formed by different multi-species mixtures of trees and crops that makes it more resilient, ease recovery from disturbances, and accelerate the successional processes (Solbrig 1994; Choi *et al.* 2008; Leakey 2012), then this system may accrue a total ecosystem goods and services greater than the output of those species if they were grown separately on equal land area (Atangana *et al.* 2014). The multi-species approach has been suggested in restoration practices (Lamb, 2011; Martínez-Garza and Howe, 2003; Palmer *et al.*, 2006, 1997). However, the mechanism on how the species diversity and the ecosystem functions is sometimes confusing (Swift *et al.*, 2004) and most of the restoration experiments have used trials-and-errors approach (Palmer *et al.*, 2006) which make it difficult to predict the optimum method in various cases. Therefore, an understanding of both the biophysical processes and the mechanisms involved in the allocation of resources is essential for the development of ecologically sound agro-forestry systems that are sustainable, economically viable, and socially acceptable (Jose *et al.*, 2009).

Soil ecosystem diversity has the same role as the aboveground species diversity in providing the services. Healthy soil ecosystem is formed by various microorganism species with specific role and function (Paul, 2014). While some plants are host for other symbiotic microorganisms, their existence and diversity are very correlated (van der Heijden *et al.*, 1998). The introduction of inoculation with mixed microorganism species is expected to restore the soil ecosystem and ameliorate the soil condition. At the same time, some microorganisms are also able to extract the contaminants from soil, which often are used in phytobial remediation technologies (Ali *et al.*, 2013; Weyens *et al.*, 2009). Degraded post-mining soil is not an ideal material for the plant growth. The physico-chemical and microbiological characteristics of the materials are too poor for optimal plant growth (Bois *et al.*, 2005; Nadeau *et al.*, 2018a, 2018b; Yonli *et al.*, 2020). Fertilization may help the plant to grow better, but it can be costly and not sustainable in ecological restoration (Nadeau *et al.*, 2016). The introduction of soil amendment could be a sustainable option supporting the plant growth, as it has been shown to improve soil properties and functions relevant to agronomic and environmental performance (Joseph and Lehmann, 2009; Woolf *et al.*, 2010). 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 hold key roles in sustaining soil health and functioning, are directly affected by the addition of biochar to soils (Ogawa, 1994; Rondon *et al.*, 2007; Steiner, 2008; Warnock *et al.*, 2007).

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.

3.4 Material and methods

3.4.1 Greenhouse mesocosm experiment

The plastic rectangular containers of 34 cm x54 cm x18 cm were used as mesocosm experiment unit. They were filled with two types of waste gold mine materials (fine tailing and waste rock). Biochar and Hydrogel amendments, micro-symbiont inoculation, and combination of tree and herbaceous crop species (HerbMix) were used. The experiment design was split-split plot with 3 blocks and 36 treatment combinations resulting in a total number of 108 experimental units. The mixture of woody species was randomly planted with Latin square arrangement as shown in *Figure 3.1*, with 8 cm spacing. The purpose of this arrangement was to give a balanced interaction for all 4 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.

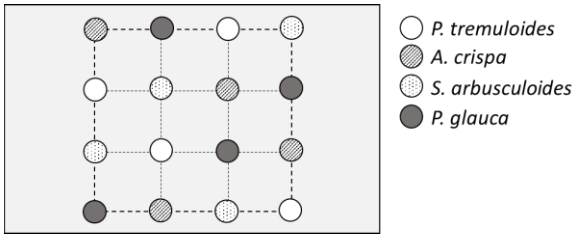

Figure 3.1 Tree seedling plantation arrangements. The seedling position is shown by colored circle (gray shading) and the color legend shows different species.

The woody plants were first propagated for one week in small 10*20 mm pellets Jiffy-7 Forestry (Stuewe & Sons, Inc., Tangent, Oregon 97389 USA). Half of the seedlings were inoculated with specific microorganisms and planted on 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 the rate of 0.0075 m^3/m^2. The hydrogel application was 20 gr /liter of water mixed with soil (Solid Rain Corp., San Diego CA, 92101).

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

3.4.1.1. *Biochar and hydrogel amendments*

The biochar used in this experiment is from maple barks (*Acer saccharum*; size fractions between 0.5 and 5 cm) produced by Award Caoutchouc & Plastique Ltée (Notre-Dame- de-Lourdes, Quebec, CA, see also Jean (2017)). Biochar is organic carbon made from biomass through pyrolysis processes. In pyrolysis the biomass is heated to temperatures between 300 and 1000 °C, under low or zero oxygen concentrations (Cha *et al.*, 2016).

The hydrogels are polymeric materials with hydrophilic structure capable of holding a large amount of water in their three-dimensional networks. They can absorb and store water hundreds of times their own weight or about 400–1500 g water per dry gram of hydrogel (Abedi-Koupai *et al.*, 2008). This water can be a reservoir for the plant on a dry session, thus increasing the plant productivity. Hydrogels also have potential effects on soil infiltration rates, density, soil structure, compaction, soil texture, aggregate stability and crust hardness (Abedi-Koupai *et al.*, 2008).

3.4.1.2. *Plant-microbial organisms*

The selected plant species were *A. viridis* subsp. *crispa*, *P. glauca*, *P. tremuloides* and *S. arbusculoides*. The four species are native to North America region and are commonly found growing in Abitibi-Témiscamingue region. Each of those species can be dominant or codominant depending on the type of habitats (Barbour and Billings, 2000). The habitat composition may change by the time with the dynamics of ecosystem and successional processes. In this experiment, we were combining all those four species at the initial stage and expecting to get the potential benefit of different species composition for accelerating the restoration processes. *P. tremuloides* and *S. arbusculoides* are fast growing species, followed by *A. viridis* subsp. *crispa*, which is also relatively fast-growing. The three species are light demanding species and considered as pioneer species. While *P. glauca* is slow growing species and considered as mid- to late-successional species, with its shade tolerant characteristics (Abrahamson, 2015). All the listed species are tolerant and adapted to poor soil and disturbed sites, and are often used for restoration and rehabilitation projects (Abrahamson, 2015; Callender *et al.*, 2016; Esser, 1992; Howard, 1996; Matthews, 1992; Nadeau *et al.*, 2018a, 2018b; Nadeau and P. Khasa, 2016), especially *A. viridis* subsp. *crispa*, which can grow well on poor soils because of its association with the nitrogen fixing actinobacteria *Frankia* spp. and mycorrhizal fungi (Roy *et al.*, 2007). Herbaceous species associations were also included as one of treatment factors. The herbaceous species included oat (*A. sativa*), red fescue (*F. rubra*), and white clover (*T. repens*). *A. sativa* is a grass species which is grown for its seeds for human consumption, and as livestock feed. The other grass species *F. rubra* is known for its tolerance on heavy metal contamination, and is often used for phytoremediation in post-mining restoration (Wong, 1982). *T. repens* is also quite tolerant on heavy metal contamination and also has the ability to fix the nitrogen with Rhizobia (Rother *et al.*, 1983). As a legume species, *T. repens* can fix the nitrogen up to 80 g N $ha^{-1}h^{-1}$ in contaminated soil (Rother *et al.*, 1983). Apart from those benefits, herbaceous species have faster turnover rate that contribute 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 on 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 on hydroseeding mixture.

3.4.2 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. Two types of waste materials were selected: fine tailing and waste rock. The field coordinates are N 48°06’38.4’’ and W 077°44’44.7’’ (fine tailing - EGL), N 48°06’20.7’’ and W 077°45’43.1’’ (waste rock - EGL), N 49°29’40.1’’ and W 076°08’49.9’’ (fine tailing - BTR), N 48°59’03.8’’ and W 075°46’18.4’’ (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 snowfall 226.2 cm. The weather information is based on data obtained between 1981 and 2010 from Canada Environment and Natural Resources website (http://climate.weather.gc.ca/climate_normals/index_e.html). Our weather station installed in June 2016 at EGL site showed the precipitation of 703.8 mm, with temperature range between -24.1° and 25.4° C and a mean annual temperature of 4.3° C.

The 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 let to evaporate over time (Aubertin *et al.*, 1996; Kossoff *et al.*, 2014). The tailings are often stored under water to reduce the contact with the atmosphere and prevents the oxidation (Kossoff *et al.*, 2014). In a dry climate, evaporation from ponded tailings water and wet tailings can lead to a concentration of salinity. The tailings in our field sites are mainly composed of biotite and Fe (Taner *et al*, 1986). The chemical analyses (Nadeau, 2015) are as follows: Sulfur (0.48 to 0.51%), Al (5500 to 6100 mg/kg), Ca (21000 to 23000 mg/kg), Fe (14000 to 16000 mg/kg), Mg (4000 to 4500 mg/kg), P (0 to 560 mg/kg), K (86 to 100 mg/kg). Zn, Mn, Cu, Mo, and Na were 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 Cyanides 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 range 10^{-4} to 10^{-5} cm/sec, with grain size <74 µm (Aubertin *et al.*, 1996), while waste rock has very high hydraulic conductivity range 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) (Kossoff *et al.*, 2014). Soil material with big particle size and high hydraulic conductivity like waste rock is not suitable for plant growth. This material has low water holding capacity and may lack water on dry periods. The silty clay soil like fine tailing with very low hydraulic conductivity is also not good either for plant growth. This silty clay can be sticky and plastic when wet and prone to drainage problems, but hard when dry (Gardner *et al.*, 1999). Both materials have very extreme physico-chemical properties which are not suitable for plant growth, the ideal soil texture being between loam and silt, with pH between 5.8-6.5 (Gardner *et al.*, 1999).

3.4.2.1. *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 combination of tree and herbaceous crop species. The plot dimension was 5 m x 8 m and tree plantation arrangement is shown in *Figure 3.2*. 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 x 20 cm, 40 cm x40 cm and 60 cm x60 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.

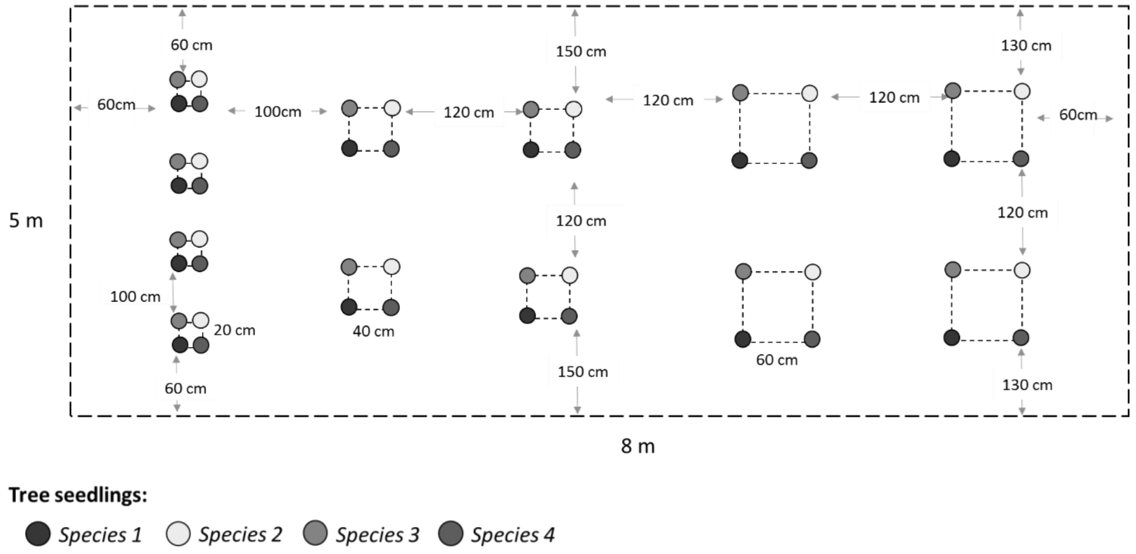

Figure 3.2. A plot showing tree seedling plantation arrangements. The seedling position is shown by the circle and the gray scale legend shows a distinct species.

The seeds were germinated on 20*32 mm pellets Jiffy-7 Forestry in the greenhouse. The pellet is 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 plantation took place in June 2016 and monitored for two growing sessions in September each year.

The herbaceous crops were planted using hydro-seeding method with commercial mulch Beno-Vert made from recycled paper (Soprema-Quebec, Quebec, CA, see also Jean (2017)). The seedling rate ratio was based on the common seedling rate of oat (*A. sativa*) as companion crop, which is 50 to 75 kg/ha (Lanini *et al.*, 1991). But here we increased the seedling rate ratio for 10% (84 kg/ha) to compensate higher mortality rate on degraded soil. For the other herbaceous species, weight ratio was adjusted for the same number of seeds as oat, 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 grams/ha of 15-30-15 fertilizer in water (40000 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 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 EGL site. The probes were installed in 15 cm and 30 cm depth on each experiment plot within the two blocks. Lysimeters were also installed on the same blocks in 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).

3.4.3 Statistical and data analyses

The collected data 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 done in the beginning of experiment (June 2016), in September 2016 for the first growing season, and in September 2017 for the second growing season. Since the first growing season had data collected in shorter time range (4 months) and can be biased due to the adaptation factors, we decided to use only growth data of the second growing season (one-year growth). Apart from that, we also excluded the height increment data because we found bias on some plants which 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 (Dean *et al.*, 2017; Pardo, 2020). The fitting uses restricted maximum likelihood (REML) method from the lme4 package (Bates *et al.*, 2014) in R software (R Core Team, 2018) for both greenhouse experiment and field trials with unbalanced data. A general statistical term with the assumption of fixed effect (α, β) and split-split plot factors ($\emptyset$, ρ, δ) is as follows (Dean *et al.*, 2017):

$$\begin{aligned} y_{dhijqrt} = {} & \mu + \theta_h + \alpha_i + (\emptyset_2)_{q_2} + (\rho_2)_{r_2} + \epsilon^{W}_{d(h)} + \beta_j + \alpha\beta_{ij} + (\emptyset_3)_{q_3} + (\emptyset_2\emptyset_3)_{q_2q_3} \\ & + (\rho_3)_{r_3} + (\rho_2\rho_3)_{r_2r_3} + \epsilon^{SS}_{ijq_3r_3(dhq_2r_2)} + \delta_t + (\beta\delta)_{jt} + \epsilon^{SS}_{t(dhijqr)}, \end{aligned}$$

$\theta_h \sim N(0, \sigma_\theta^2)$, $\epsilon_{d(h)}^W \sim N(0, \sigma_W^2)$, $\epsilon_{ijq_3r_3(dhq_2r_2)}^S \sim N(0, \sigma_S^2)$, $\epsilon_{t(dhijqr)}^{SS} \sim N(0, \sigma_{SS}^2)$

θ_h's, $\epsilon_{d(h)}^W$'s, $\epsilon_{ijq_3r_3(dhq_2r_2)}^{SS}$'s, $\epsilon_{t(dhijqr)}^{SS}$'s are mutually independent.
d, *h*, *i*, *j*, *q*, *r*, *t* are observation level for each corresponded factors.

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 is inoculation nested under supplements, under tailings, under blocks. The statistical term for lmer() method on R software is as follows (Dean *et al.*, 2017; Pardo, 2020):

*Y = Tailings * Supplement * Inoculation * HerbMix + (1|Block/Tailings /Supplement/Inoculation) +* ϵ

Where *Y* is dependent variable for plant biomass and shoot:root ratio.

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

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

*Growth = Species * Tailings * InitDiameter * Biochar * Inoculation * HerbMix + (1|Location/Tailings/Block/Biochar/Inoculation/HerbMix) +* ϵ

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| Location/Block/Biochar/Inoculation/HerbMix) +* ϵ

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

3.5 Results

3.5.1 Greenhouse experiment

The woody species were not growing 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 on herbaceous crop species but total above and belowground biomass was for all species including the woody species.

The total biomass was dominated by *A. sativa* and seems to be higher in fine tailing than in waste rock material, as shown in Figure 3.3. 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 that allows 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.

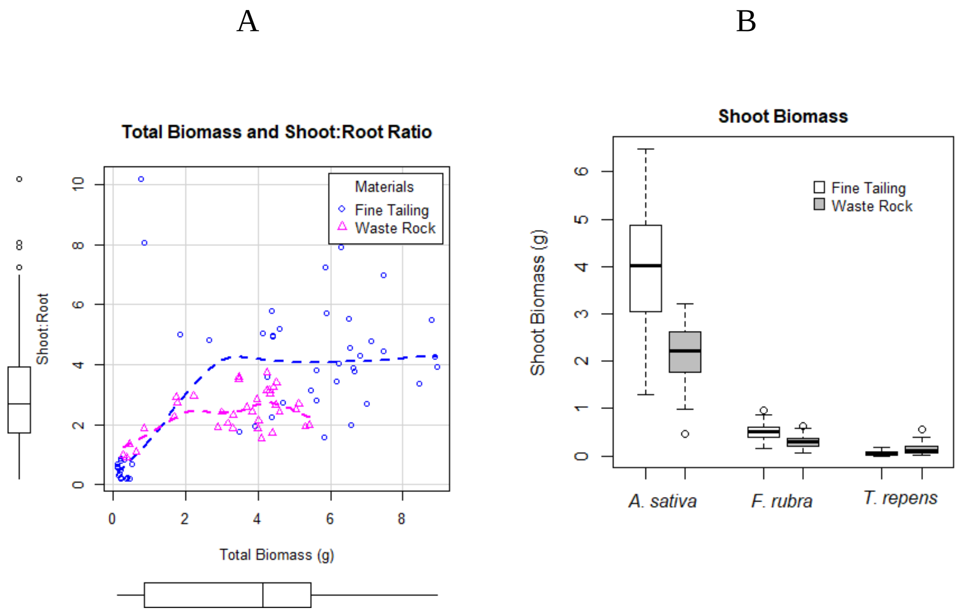

Figure 3.3 (A) Dry weight biomass and shoot:root ratio of herbaceous and woody species compared between fine tailing and waste rock growth materials after 3 months of growth. The loess regression

curve shows a tendency of constant shoot:root ratio on bigger plant (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 for fixed effect is shown in Table 3.1 and random effect in Table 3.2. The total biomass has a significant interaction between herbaceous mixing with tailings as well as herbaceous mixing and amendments. The shoot:root ratio showed to be lower on waste rock material (Figure 3.3), which could be an indicator of higher limitation of nutrient compared to fine tailing material. But statistically we found no correlation between the shoot:root ratio with all treatment factors in our experiment (Table 3.1), although in Figure 3.3 we can see some differences between tailing materials. The stunted seedlings of the tree treatment (perennial species) with low survival rate, caused a high variability in shoot:root ratio, thus the statistical results for the tree treatment should be interpreted cautiously.

Table 3.1 The ANOVA P-value for total biomass, shoot biomass of A. sativa, F. rubra, T. repens, and shoot:root ratio.

Source	Aboveground biomass of			Total biomass	Shoot:root ratio
	A. sativa	*F. rubra*	*T. repens*		
Tailings	0.000 ***	0.123	0.136	0.000 ***	0.112
Amendement	0.001 **	0.001 **	0.526	0.001 **	0.165
Symbiotic	0.002 **	0.175	0.003 **	0.001 **	0.411
HerbMix	0.000 ***	0.005 **	0.004 **	0.000 ***	0.429
Tailings:Amendement	0.123	0.002 **	0.314	0.075	0.761
Tailings:Symbiotic	0.649	0.089	0.269	0.664	0.511
Amendement:Symbiotic	0.700	0.182	0.120	0.921	0.190
Tailings:HerbMix	0.421	0.738	0.228	0.000 ***	0.394
Amendement:HerbMix	0.008 **	0.172	0.034 *	0.001 **	0.194
Symbiotic:HerbMix	0.226	0.979	0.063	0.887	0.089
Tailings:Amendement:Symbiotic	0.419	0.365	0.806	0.481	0.280
Tailings:Amendement:HerbMix	0.142	0.383	0.300	0.381	0.881
Tailings:Symbiotic:HerbMix	0.556	0.447	0.108	0.666	0.294
Amendement:Symbiotic:HerbMix	1.000	0.139	0.751	0.250	0.650
Tailings:Amendement:Symbiotic:HerbMix	0.548	0.091 .	0.785	0.101	0.308

*Significance codes: $P < 0.001$ '***', $P < 0.01$ '**', $P < 0.05$ '*'*

Table 3.2 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.

Groups	No. of Obs.	Aboveground biomass of			Total biomass	Shoot:root ratio
		A. sativa	*F. rubra*	*T. repens*		
Inoculation:(Supplement:(Tailings:Block))	36	0.000	0.000	0.627	0.000	0.000
Supplement:(Tailings:Block)	18	0.058	0.069	0.000	0.013	0.000
Tailings:Block	6	0.000	0.275	0.772	0.000	0.200
Block	3	0.129	0.140	0.000	0.120	0.084
Residual		0.260	0.243	0.775	0.208	0.277

Figure 3.4 shows some interaction effects of the main factors on total biomass was significant but we could not find others the slight significant effect on the interactions between some main effects at $P < 0.05$ (see table 3.1). Herein the biochar amendment had negative effect on total biomass while hydrogel showed slightly positive effect but not significant at $P < 0.05$.

The measured soil temperature using the LICOR temperature sensor showed that the biochar treatment has slightly higher temperature (data not shown). Our assumption is that biochar might increase the soil surface temperature (by lowering the soil albedo), which may accelerate the evaporation and limiting the available water for the plant.

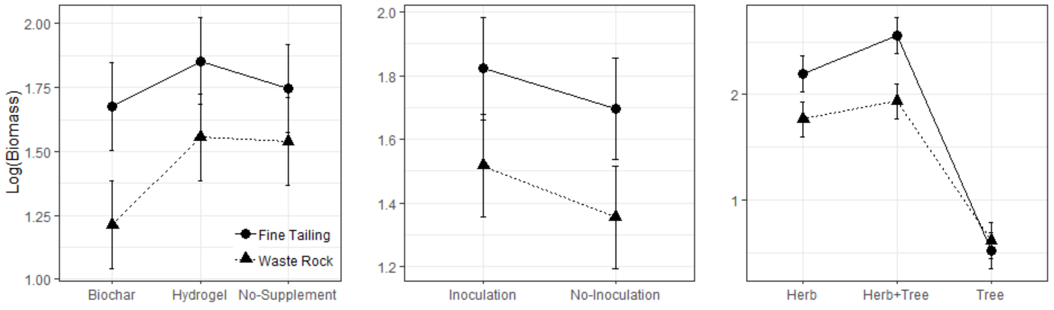

Figure 3.4 The effect of materials, soil amendments, microbial inoculation and plantation method factors on total biomass and shoot:root ratio. The error bars represent 95% confidence interval (CI) of means.

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 helps the plant accelerate the 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. But the analysis on 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. On a separate analysis, we found that the benefit of tree species addition is higher with biochar treatment. Thus, suggesting that the inclusion of tree species might help on 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.

3.5.2 The field trials

The plant mortality was high on waste rock site (60% at Sigma and 80% at Metanor), while fine tiling showed better survival rate (2% and 10% mortality on Sigma and Metanor sites, respectively) during the first month after initial plantation. Surprisingly, the rest of plants on waste rock were able to survive until second growing session with mortality less than 10%. Thus, we assumed that the cause of high mortality (up to 80%) was only during the initial adaptation to transplant shock, which could be due to the harsh climate and worst soil conditions on the waste rock material. The spacing analysis was then excluded for the waste rock materials as it did no longer have 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 variance table was showed on Table 3.3 and Table 3.5, while the standard deviation for random effect is shown on Table 3.4 and Table 3.6. Table 3.3 is the analysis of variance for all data on waste rock and fine tailing but without spacing factor, while Table 3.5 is the analysis of variance for fine tailing only with the inclusion of spacing factor.

Table 3.3 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.

Source	Sum Sq	DF	F value	Pr(>F)	
Species	5.760	3	1.030	0.378	
Biochar	5.027	1	2.696	0.101	
Inoculation	5.751	1	3.085	0.079	
Tailings	3.100	1	1.663	0.215	
HerbMix	0.270	1	0.145	0.704	
InitDiameter	28.560	1	15.318	0.000	***
Species:Biochar	4.930	3	0.882	0.450	
Species:Inoculation	35.145	3	6.284	0.000	***
Biochar:Inoculation	5.386	1	2.889	0.089	
Species:Tailings	3.689	3	0.660	0.577	
Biochar:Tailings	4.276	1	2.293	0.130	
Inoculation:Tailings	8.169	1	4.381	0.037	*
Species:HerbMix	1.624	3	0.290	0.832	
Biochar:HerbMix	0.201	1	0.108	0.743	
Inoculation:HerbMix	17.020	1	9.129	0.003	**
Tailings:HerbMix	1.122	1	0.602	0.438	
Species:InitDiameter	25.883	3	4.628	0.003	**
Biochar:InitDiameter	3.250	1	1.743	0.187	
Inoculation:InitDiameter	2.403	1	1.289	0.256	
Tailings:InitDiameter	11.263	1	6.041	0.014	*
HerbMix:InitDiameter	1.484	1	0.796	0.372	
Species:Biochar:Inoculation	7.171	3	1.282	0.279	
Species:Biochar:Tailings	5.221	3	0.933	0.424	
Species:Inoculation:Tailings	7.982	3	1.427	0.233	
Biochar:Inoculation:Tailings	8.304	1	4.454	0.035	*
Species:Biochar:HerbMix	1.737	3	0.311	0.818	
Species:Inoculation:HerbMix	27.813	3	4.973	0.002	**
Biochar:Inoculation:HerbMix	5.302	1	2.844	0.092	
Species:Tailings:HerbMix	0.975	3	0.174	0.914	
Biochar:Tailings:HerbMix	1.026	1	0.550	0.458	
Inoculation:Tailings:HerbMix	8.164	1	4.379	0.037	*
Species:Biochar:InitDiameter	3.895	3	0.696	0.554	
Species:Inoculation:InitDiameter	22.732	3	4.064	0.007	**
Biochar:Inoculation:InitDiameter	4.528	1	2.429	0.119	
Species:Tailings:InitDiameter	4.659	3	0.833	0.476	
Biochar:Tailings:InitDiameter	4.380	1	2.349	0.125	
Inoculation:Tailings:InitDiameter	13.223	1	7.092	0.008	**
Species:HerbMix:InitDiameter	1.438	3	0.257	0.856	
Biochar:HerbMix:InitDiameter	0.543	1	0.291	0.590	
Inoculation:HerbMix:InitDiameter	10.533	1	5.650	0.018	*

Tailings:HerbMix:InitDiameter	3.206	1	1.720	0.190	
Species:Biochar:Inoculation:Tailings	9.056	3	1.619	0.183	
Species:Biochar:Inoculation:HerbMix	22.172	3	3.964	0.008	**
Species:Biochar:Tailings:HerbMix	1.562	3	0.279	0.840	
Species:Inoculation:Tailings:HerbMix	11.877	3	2.124	0.095	
Biochar:Inoculation:Tailings:HerbMix	0.201	1	0.108	0.742	
Species:Biochar:Inoculation:InitDiameter	4.923	3	0.880	0.451	
Species:Biochar:Tailings:InitDiameter	5.946	3	1.063	0.364	
Species:Inoculation:Tailings:InitDiameter	8.433	3	1.508	0.211	
Biochar:Inoculation:Tailings:InitDiameter	2.546	1	1.365	0.243	
Species:Biochar:HerbMix:InitDiameter	3.230	3	0.577	0.630	
Species:Inoculation:HerbMix:InitDiameter	24.610	3	4.400	0.004	**
Biochar:Inoculation:HerbMix:InitDiameter	2.582	1	1.385	0.239	
Species:Tailings:HerbMix:InitDiameter	2.438	3	0.436	0.727	
Biochar:Tailings:HerbMix:InitDiameter	1.380	1	0.740	0.390	
Inoculation:Tailings:HerbMix:InitDiameter	8.491	1	4.554	0.033	*
Species:Biochar:Inoculation:Tailings:HerbMix	6.679	3	1.194	0.310	
Species:Biochar:Inoculation:Tailings:InitDiameter	4.024	3	0.720	0.540	
Species:Biochar:Inoculation:HerbMix:InitDiameter	25.259	3	4.516	0.004	**
Species:Biochar:Tailings:HerbMix:InitDiameter	1.716	3	0.307	0.821	
Species:Inoculation:Tailings:HerbMix:InitDiameter	12.417	3	2.220	0.084	.
Biochar:Inoculation:Tailings:HerbMix:InitDiameter	0.048	1	0.026	0.873	
Species:Biochar:Inoculation:Tailings:HerbMix:InitDiameter	7.498	3	1.340	0.259	

*Significance codes: P < 0.001 '***', P < 0.01 '**', P < 0.05 '*'*

Table 3.4 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.

Groups	No. of Obs.	Variance	Std.Dev.
HerbMix:(Inoculation:(Biochar:(Block:(Tailings:Location))))	122	0.051	0.226
Inoculation:(Biochar:(Block:(Tailings:Location)))	63	0.052	0.228
Biochar:(Block:(Tailings:Location))	32	0.000	0.000
Block:(Tailings:Location)	16	0.001	0.030
Tailings:Location	4	0.031	0.177
Location	2	0.000	0.000
Residual		1.864	1.365

Table 3.5 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.

Source	Sum Sq	DF	F value	Pr(>F)	
Species	21.944	3	3.946	0.008	**
InitDiameter	5.209	1	2.810	0.094	
Biochar	0.694	1	0.374	0.541	
Inoculation	0.054	1	0.029	0.865	
HerbMix	0.333	1	0.180	0.672	
Spacing	2.663	2	0.718	0.488	
Species:InitDiameter	15.171	3	2.728	0.043	*
Species:Biochar	2.786	3	0.501	0.682	
InitDiameter:Biochar	0.186	1	0.101	0.751	
Species:Inoculation	19.401	3	3.489	0.015	*
InitDiameter:Inoculation	5.091	1	2.746	0.098	
Biochar:Inoculation	0.000	1	0.000	0.994	
Species:HerbMix	3.028	3	0.544	0.652	
InitDiameter:HerbMix	0.288	1	0.156	0.693	
Biochar:HerbMix	0.862	1	0.465	0.496	
Inoculation:HerbMix	1.853	1	1.000	0.318	
Species:Spacing	8.036	6	0.722	0.632	
InitDiameter:Spacing	5.002	2	1.349	0.260	
Biochar:Spacing	2.981	2	0.804	0.448	
Inoculation:Spacing	3.679	2	0.992	0.371	
HerbMix:Spacing	17.429	2	4.701	0.009	**
Species:InitDiameter:Biochar	6.319	3	1.136	0.333	
Species:InitDiameter:Inoculation	10.523	3	1.892	0.129	
Species:Biochar:Inoculation	1.750	3	0.315	0.815	
InitDiameter:Biochar:Inoculation	1.623	1	0.876	0.350	
Species:InitDiameter:HerbMix	5.297	3	0.952	0.414	
Species:Biochar:HerbMix	3.629	3	0.653	0.581	
InitDiameter:Biochar:HerbMix	0.613	1	0.331	0.565	
Species:Inoculation:HerbMix	9.528	3	1.713	0.162	
InitDiameter:Inoculation:HerbMix	0.147	1	0.079	0.778	
Biochar:Inoculation:HerbMix	4.057	1	2.189	0.139	
Species:InitDiameter:Spacing	8.470	6	0.762	0.600	
Species:Biochar:Spacing	4.014	6	0.361	0.904	
InitDiameter:Biochar:Spacing	1.813	2	0.489	0.613	
Species:Inoculation:Spacing	15.594	6	1.402	0.210	
InitDiameter:Inoculation:Spacing	4.324	2	1.166	0.312	
Biochar:Inoculation:Spacing	3.091	2	0.834	0.435	
Species:HerbMix:Spacing	13.021	6	1.171	0.319	

InitDiameter:HerbMix:Spacing	17.884	2	4.824	0.008	**
Biochar:HerbMix:Spacing	0.323	2	0.087	0.917	
Inoculation:HerbMix:Spacing	0.070	2	0.019	0.981	
Species:InitDiameter:Biochar:Inoculation	1.829	3	0.329	0.805	
Species:InitDiameter:Biochar:HerbMix	3.504	3	0.630	0.596	
Species:InitDiameter:Inoculation:HerbMix	5.815	3	1.046	0.371	
Species:Biochar:Inoculation:HerbMix	6.229	3	1.120	0.340	
InitDiameter:Biochar:Inoculation:HerbMix	4.693	1	2.532	0.112	
Species:InitDiameter:Biochar:Spacing	3.955	6	0.356	0.907	
Species:InitDiameter:Inoculation:Spacing	12.619	6	1.135	0.340	
Species:Biochar:Inoculation:Spacing	10.646	6	0.957	0.453	
InitDiameter:Biochar:Inoculation:Spacing	1.588	2	0.428	0.652	
Species:InitDiameter:HerbMix:Spacing	13.069	6	1.175	0.317	
Species:Biochar:HerbMix:Spacing	3.440	6	0.309	0.932	
InitDiameter:Biochar:HerbMix:Spacing	1.258	2	0.339	0.712	
Species:Inoculation:HerbMix:Spacing	16.563	6	1.489	0.178	
InitDiameter:Inoculation:HerbMix:Spacing	0.034	2	0.009	0.991	
Biochar:Inoculation:HerbMix:Spacing	3.937	2	1.062	0.346	
Species:InitDiameter:Biochar:Inoculation:HerbMix	6.493	3	1.167	0.321	
Species:InitDiameter:Biochar:Inoculation:Spacing	9.475	6	0.852	0.530	
Species:InitDiameter:Biochar:HerbMix:Spacing	7.393	6	0.665	0.678	
Species:InitDiameter:Inoculation:HerbMix:Spacing	17.857	6	1.605	0.142	
Species:Biochar:Inoculation:HerbMix:Spacing	19.573	6	1.760	0.104	
InitDiameter:Biochar:Inoculation:HerbMix:Spacing	4.111	2	1.109	0.330	
Species:InitDiameter:Biochar:Inoculation:HerbMix:Spacing	20.132	6	1.810	0.093	

*Significance codes: $P < 0.001$ '***', $P < 0.01$ '**', $P < 0.05$ '*'*

Table 3.6 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.

Groups	No. of Obs.	Variance	Std.Dev.
HerbMix:(Inoculation:(Biochar:(Block:Location)))	64	0.044	0.211
Inoculation:(Biochar:(Block:Location))	32	0.023	0.153
Biochar:(Block:Location)	16	0	0
Block:Location	8	0	0
Location	2	0.062	0.249
Residual		1.854	1.362

We found interactions between all factors ($P < 0.01$) and it is difficult to interpr*et al*l the interactions in 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 3.5

shows the marginal interaction effect for each plant species and tailing with biochar treatment on plant diameter increments.

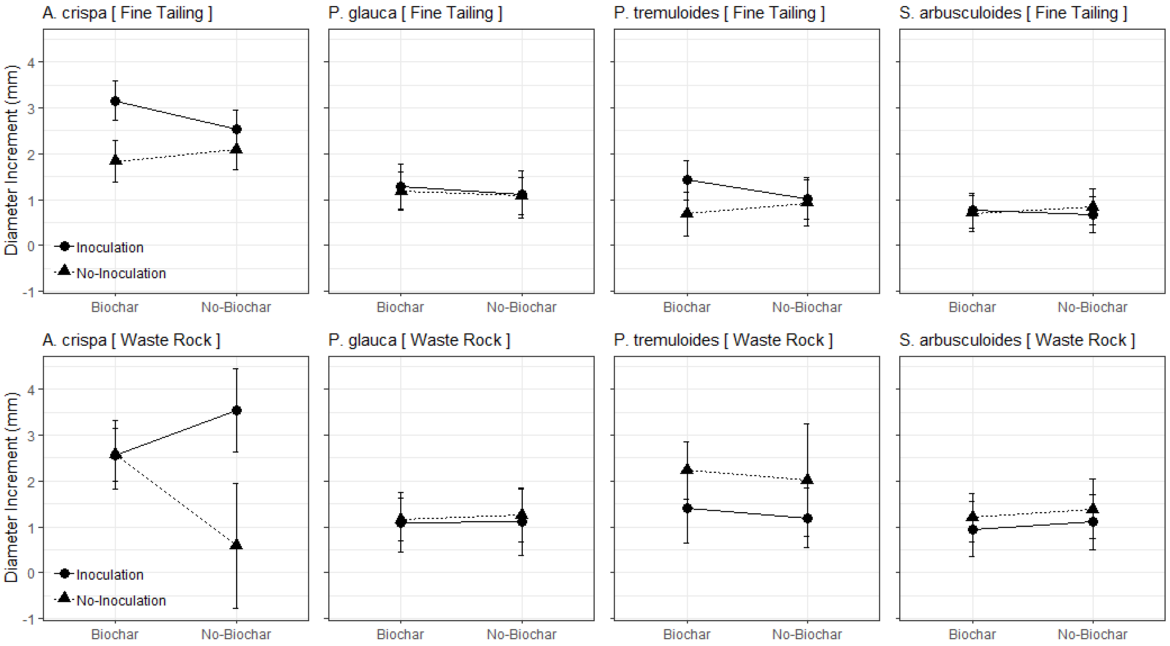

Figure 3.5 The marginal interaction effect of plant species, tailing, biochar and inoculation treatment on plant diameter increments. The error bars represent 95% confidence interval (CI) of means.

The marginal interaction effect in Figure 3.5 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 showing no significant effect on *P. glauca* and *S. arbusculoides*. While 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 3.5). But the effect was opposite on waste rock for *A. viridis subsp. crispa*. The interaction of biochar and inoculation seem to have no significant effect on the rest of the species.

The effect of biochar was found to be negative in greenhouse experiment on herbaceous biomass in both fine tailing and waste rock tailings (Figure 3.4). Thus, the biochar effect will vary depending on plant species and environment. Figure 3.6 showed that biochar effect also differed for various plant spacings. The various effect also applies to inoculation treatment on different plant species, 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.

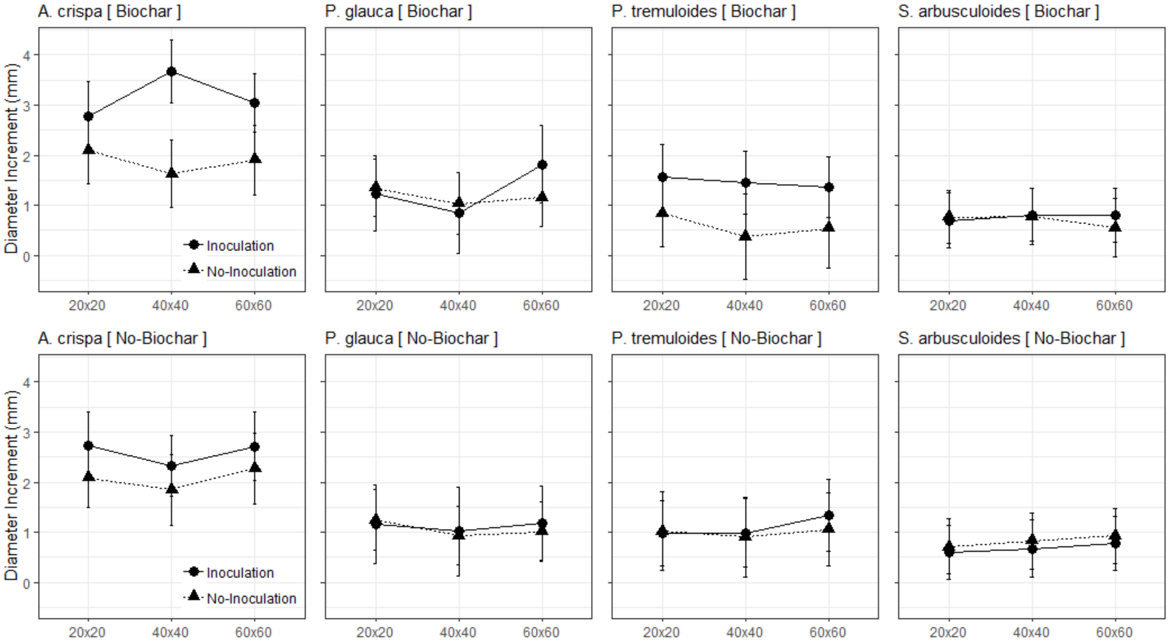

Figure 3.6 The interaction effect of biochar, inoculation and spacing treatments in fine tailing site. The error bars represent 95% confidence interval (CI) of means.

The spacing treatment showed unexpected results as shown in Figure 3.6. The plant mixing with highest density or 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 of other interactions, the growth rate showed to be decreasing from the largest spacing (60 x 60 cm) to the middle spacing (40 x 40 cm) and increasing again on the smallest spacing (20 x 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 increasing growth rate from the largest spacing to the intermediate spacing and 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 3.6). The positive response of *A. viridis subsp. crispa* and *P. tremuloides* with the biochar and inoculation treatments at 40 x 40 cm spacing showed the importance of density or spacing configuration on the interaction of the treatments (Figure 3.6). The 40 x 40 cm spacing was the optimum spacing for *A. viridis subsp. crispa* and *P. tremuloides* where the balance is acquired between competition and facilitation.

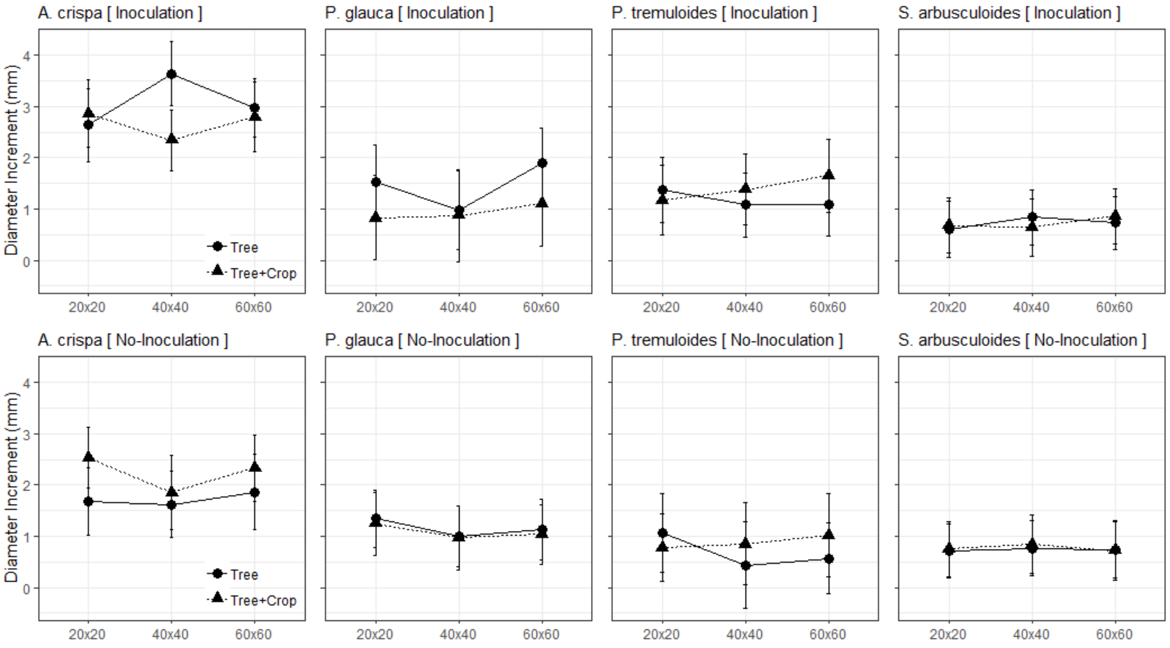

Figure 3.7 The interaction effect of inoculation, tree-crop and spacing treatments in fine tailing site. The error bars represent 95% confidence interval (CI) of means.

The addition of herbaceous crop plants did not show to have a significant effect on woody plant growth (Figure 3.7), 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 cover crop in this case, which maintain the evaporation and soil surface temperature around the woody plants. But the plant response changed when the inoculation was added. Thereby improving the herbaceous growth and the competitive effect on the perennial woody plant.

3.6 Discussion

The physical characteristics of the fine tailing with very high moisture retention (Blowes, 1997) and very low hydraulic conductivity (Aubertin *et al.*, 1996) was not ideal for the plant growth. The particle size was between clay and silt, which has high permanent wilting point (± 27%) (Saxton and Rawls, 2006). Soil moisture at 15 cm below the surface was always below 30% for the whole year based on our measurements. Although the site has 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 have inadequate characteristics for the plant growth in both greenhouse and field trials. The tree species exhibited better survival than herbaceous species in the field trial after two growing session than in the greenhouse trial. This can be explained by the fact that the woody perennial species used in the field trails are native and ecologically well adapted to the boreal Forest of Abitibi-Témiscamingue region while the herbaceous species were allochthonous species to the region. These results corroborate those of Nadeau *et al.* (2018a, 2018b) who showed the importance on 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 survivability and adaptability of tree species. While the biochar and inoculation treatments did not to influence plant survivability, but it had the effect on the biomass yield productivity.

3.6.1 Biochar amendment

Biochar is a highly stable and rich of carbon, which is a potential carbon sink in relation to climate change mitigation (Jeffery *et al.*, 2011). Biochar is also known as soil amendment for improving the soil properties and functions in agronomic applications (Jeffery *et al.*, 2011; Lehmann *et al.*, 2011; Sohi *et al.*, 2010). It has the capacity to enhance the water and nutrient retention and improves soil structure and drainage (Jeffery *et al.*, 2011). There is evidence of biochar effect on microbial activity and plant symbiosis which improve crop productivity (Jeffery *et al.*, 2011; Lehmann *et al.*, 2011). The possible mechanisms involved include the immobilization of plant available N, the mineralization of labile, high C–to–N fractions of biochar into microbial biomass (Sohi *et al.*, 2010). 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 (Tarkka and Frey-Klett, 2008; Warnock *et al.*, 2007).

In opposite to those reports above, our greenhouse experiment with biochar amendment showed a negative effect on total biomass yield (Figure 3.4). In fact, the plants did not benefit from the improvement of 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 field trial, the biochar showed a positive effect, especially on *A. viridis subsp. crispa* and *P. tremuloides* (Figure 3.6). But surprisingly the effect become negative on bigger spacing (60 x 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 of microclimate condition on higher density (Asmara *et al.*, 2020; Corbin and Holl, 2012; Courchamp *et al.*, 2008) (see also Chapter 2).

There was some other effect of biochar amendment on soil which is the reduction of soil albedo (Verheijen *et al.*, 2013). The lower albedo of biochar can make the soil warmer, which can make more soil water evaporation compared to the higher albedo, resulting in negative effect on plant growth. Some interesting results on the effect of biochar on soil temperature in temperate zone has shown that it can increase the average soil temperature, but it has lower temperature on hottest day in a year (Zhang *et al.*, 2013). 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 (Curiel Yuste *et al.*, 2007). The improvement of microbial activity on a warmer soil could be another interaction mechanism between biochar and microbial inoculation in our field trial experiments.

3.6.2 Microbial inoculation

A healthy soil ecosystem is formed by various microbial species with specific role and function (Paul, 2014). We believe that soil ecosystem biodiversity is as important as aboveground biodiversity. We also believe that above- and below-ground biodiversity is highly correlated as some microorganisms may require specific host plants (van der Heijden *et al.*, 1998). Thus, providing the mixture of root inoculants for the plants is expected to introduce and increase belowground 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 (Fortin *et al.*, 2015; Smith and Read, 2009; Swift and Anderson, 1994).

The inoculation treatment showed a positive effect on plant growth in greenhouse experiment. It had a slightly positive interaction with biochar, although it was not significant. The inoculation was beneficial for the plant growth in both fine tailing and waste rock Tailings. Since the mining waste tailings are mostly deprived from beneficial microorganisms (Bois *et al.*, 2005; Yonli *et al.*, 2020), the inoculation with beneficial microbes was shown as a good way for introducing beneficial symbiotic microorganisms in the these challenging reclamation tailings as reported in other studies (among other Bissonnette *et al.*, 2014; Nadeau *et al.*, 2018a, 2018b; Onwuchekwa *et al.*, 2014).

The field trials showed the 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 the efficiency of different microsymbionts (Nadeau *et al.*, 2018a, 2018b, 2016). The symbiotic relationship can range from parasitism to true mutualism depending on the microsymbiont, the plant host and the soil fertility (Franklin *et al.*, 2014; Smith *et al.*, 2009; Smith and Read, 2009). *Alnus viridis subsp. crispa* as an ectomycorrhizal plant benefits symbiotic mutualism from both nitrogen fixing *Frankia* actinomycete and mycorrhizal fungi (Roye *et al.*, 2007). *P. tremuloides* is not a nitrogen fixing plant but can form both ectomycorrhizal and arbuscular mycorrhizal (Khasa *et al.*, 2002) and benefit from co-occurence mycorrhizal network with *A. viridis subsp. crispa* (Bücking *et al.*, 2016; Fellbaum *et al.*, 2014; Walder *et al.*, 2012). 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 soil amendment which provides a good environment for mycorrhiza colonization (Anderson *et al.*, 2011; Lehmann *et al.*, 2011; Robertson *et al.*, 2012). But the direct mechanism on how it affects the mycorrhizal dynamics is still unclear (Lehmann *et al.*, 2011). The known mechanism in correlation with mycorrhiza is through soil chemical and physical alteration (Lehmann *et al.*, 2011; Sohi *et al.*, 2010). Another possible mechanism is through the soil temperature stabilization by the reduction of soil albedo, as discussed above. In fact, the microorganisms are known to be sensitive to soil temperature and microclimate conditions (Curiel Yuste *et al.*, 2007; Graham *et al.*, 1982; Hasselquist *et al.*, 2016; Hawkes *et al.*, 2008; Shukla *et al.*, 2009).

3.6.3 Mixed system interactions

The field trials showed a positive effect of herbaceous crop on tree species without inoculation treatment. One of the reasons for this finding is the well-known role of cover crops on reducing the evapotranspiration (Snapp *et al.*, 2005). The herbaceous crops became disadvantageous and competitive to the woody perennial species when the inoculation treatment was applied (Figure 3.7). The literature on the use of bioinoculants in agroforestry systems is very scanty (Araujo *et al.*, 2012). 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 to improve nutrient availability for plants while reducing the use inorganic or organic fertilizers, pesticides and water. More research is needed in that area in order to develop a broad conceptual framework and methodology that is supported by robust scientific data for large-scale of bioinoculants in 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 (Brooker *et al.*, 2007; Courchamp *et al.*, 2008; Holmgren *et al.*, 1997). The positive effect on higher density could be explained by the belowground 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 (Curiel Yuste *et al.*, 2007; Zhang *et al.*, 2013).

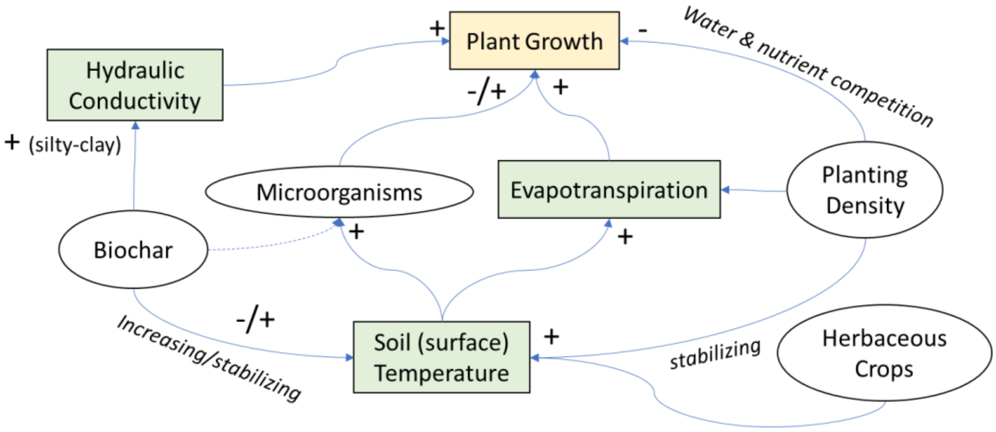

Figure 3.8 Hypothetical interactions between components factors in restoration trial experiment. The interactions are complex, and all the factors can contribute to plant growth dynamics.

We found that the interactions between components in mixed systems were not straight forward and require a comprehensive scenario for the intervention of the system. Figure 3.8 shows the ecophysiological complexity of plant responses to the different interactions between the factors that we have studied in our experiment. The addition of biochar, inoculation treatment, and herbaceous crops could improve the plant growth and at the same time it can have negative effect. The planting density treatment is not just affecting the plant competition, but also altering 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 also other elements that need to be considered in the modeling scenario.

3.7 Conclusion

Soil temperature, soil cover, evaporation, and evapotranspiration affect soil water availability. Therefore, 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, 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 soil solution. The multispecies approach has shown some advantages in our restoration experiment. Mixing the species in early stage of plantation proved to have no significant effect on spacing competition, although the outcome can be different in the later stages when the plant is getting bigger and both aboveground and below-ground competitions occur. At the early stage we found a positive balance between plant competition and microclimate improvement on high density plantation. Other 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 are expecting to find more interactions between the species through their function on the nutrient cycling and successional dynamics.

The positive effect of plant density confirms the “Allee” effect that showed the benefit of living in groups for inducing the facilitation within the individuals (Courchamp *et al.*, 2008). The practical implementation of high-density plantation can be very costly. The proposed “Nucleation” method introduced by Corbin and Holl (2012) could be a low cost alternative. The seedlings are planted in patches or “islands” as to facilitate forest recovery that is less expensive than planting large areas. But a study in tropical forest restoration in Costa Rica has highlighted the importance of broad spatial replicated studies to account for high variability and make generalizable restoration recommendations (Holl *et al.* 2011). The improvement of microclimate on high density cluster planting is known to increase survivability of the seedlings (Bertoncello *et al.*, 2016; Corbin *et al.*, 2016; Corbin and Holl, 2012). The combination with other enabling biotechniques such as microbial inoculation and biochar amendment may improve the whole successional processes. Reinstalling the biological life through microbial inoculation of seedlings outplanted in reconstructed anthroposols after mining operations has shown successful plant growth and health; and improved soil quality (Bissonnette *et al.*, 2014; Callender *et al.*, 2016; Lefrançois *et al.*, 2010; Nadeau *et al.*, 2018a, 2018b; Onwuchekwa *et al.*, 2014; Quoreshi *et al.*, 2005; Roy *et al.*, 2007; Yonli *et al.*, 2020). Biochar has a capacity to increase the hydraulic conductivity on the soil or tailings with very fine grained size like 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 like waste rock (Kammann and Graber, 2015; Lehmann *et al.*, 2011; Sohi *et al.*, 2010). The biochar also reduce the soil albedo which may increase the average soil surface temperature (Verheijen *et al.*, 2013; Zhang *et al.*, 2013). Warmer soil temperature can have negative effect on some ecosystems, but it seems to be advantageous for colder climate zone as warmer soil temperature may increase the soil microbial activity and accelerate the plant recovery processes (Curiel Yuste *et al.*, 2007).

The microclimate improvement is suggested to be a focus on the initial plantation, along with planting configuration and multispecies approach. In the long term the nutrient cycle, phytoremediation processes and successional dynamics would be some other aspects that are also important. The combination of annual and perennial plants in agroforestry systems can be applied for accelerating the nutrient cycle and successional processes for forest recovery of severely disturbed ecosystems (Vieira *et al.*, 2009). The herbaceous crops serve as cover crops for supporting the other woody plants (Bodner *et al.*, 2007; Snapp *et al.*, 2005). We believe that this multispecies and multifunctional approach can be advantageous for ecological restoration projects of mining sites.

General conclusion

The ecological restoration is based on principles focusing on multispecies approach, phytoremediation and facilitation. Agroforestry system is recommended as one option for the restoration practices considering the future increment on land requirement and food demands (Nair and Garrity, 2012). High intensive management in agroforestry and active restoration can be suggested as nature based solutions for accelerating the successional processes in ecological restoration (Bechara *et al.*, 2016; Méndez *et al.*, 2008). But the trade-offs in operation costs can be higher in intensive management or active restoration. Several methods such as applied nucleation as a forest restoration strategy can reduce the implementation costs (Corbin and Holl, 2012) and multiple ecosystem services provided by agroforestry systems can also be considered as additional return benefits.

The plantation strategy and species selection are also another challenge for multispecies principles. The nelder plot experiment has given a view on how the species were interacting along a gradient density. Combination of species had a neutral effect (neither advantages nor disadvantages) compared to single species situations on the waste rock tailings. We also found a positive effect of density on early growth of plantation which suggested that microclimate improvement played a role in the fine tailing substrate.

Fast growing species *P. tremuloides* and *S. arbusculoides* were as competitive as the other slower growing species. Thus, we found no dominant species within the selected species. While the interactions in the initial stage was dominated by belowground competition, mixing the species did not alter much the overall performance of the plants. Although the outcome may change as the plants get bigger and involve both below-ground and above-ground competing factors.

The facilitative effect was shown in fine tailing with bigger yield on higher density for *P. glauca* and *S. arbusculoides* with net positive effect of competition and facilitation. The positive effect of planting density confirms the “Allee” effect which shows the benefit of living in groups for inducing the facilitation within the individuals (Courchamp *et al.*, 2008). The restoration method with high density clustered plantation focusing on microclimate improvement was known to increase survivability of the seedlings (Bertoncello *et al.*, 2016; Corbin *et al.*, 2016; Corbin and Holl, 2012).

The microclimate improvement is suggested to be a focus on the initial plantation, along with planting configuration and mixed species. The suggested method on this experiment using modified nelder plot design and relative competition effect (RCE) were able to show specific interaction between species combination and may further be used for plant interaction modeling. While specific leaf area (SLA) was shown quite sensitive to spacing density, further research of its trade off on aboveground competition at later stages could be interesting. We believe that RCE and net interaction effect may change over time and successional stages.

The multispecies approach has shown some advantages in our field trial experiment. Some facilitative effect was shown in the inoculation treatment between *A. viridis* subsp. *crispa* and *P. tremuloides. A. viridis* subsp. *crispa* seems to become a nurse species for *P. tremuloides* through mycorrhizal networks (Simard *et al.*, 2012). The addition of herbaceous crops shows a positive effect on the perennial plant growth rate through their function as cover crops. In the long term we can expect to find more interactions between the species through their function on the nutrient cycle and successional dynamics.

The positive effect of plant density was also shown in our field trials. This was confirming the results from our nelder plot experiment with gradient density (Asmara *et al.*, 2020, Chapter 2). The mechanism is believed to be an improvement in microclimate condition around higher density plantation. This improvement of microclimates on high density clustered plantation in other experiments is known to increase survivability of the seedlings (Bertoncello *et al.*, 2016; Corbin *et al.*, 2016; Corbin and Holl, 2012).

Biochar has a capacity to increase the hydraulic conductivity on the soil or tailings with very fine grained size like fine tailing waste tailings and on the same time it also able to reduce the hydraulic conductivity on the tailings with large particle size like waste rock (Kammann and Graber, 2015; Lehmann *et al.*, 2011; Sohi *et al.*, 2010). The biochar also reduce the soil albedo which may increase the average soil surface temperature (Verheijen *et al.*, 2013; Zhang *et al.*, 2013). Warmer soil temperature can have negative effect on some ecosystems, but it seems to be advantageous in boreal ecosystems as warmer soil temperature may increase the microbial activity and accelerate the phytoremediation processes (Curiel Yuste *et al.*, 2007).

The microclimate improvement is suggested to be a focus on the initial plantation, along with planting configuration and multispecies approach. In the long term the nutrient cycle, phytoremediation processes and successional dynamics would be some other aspects that are also important to look at. The combination of annual and perennial plants as in agroforestry systems and practices can be applied for accelerating the nutrient cycle and successional processes (Vieira *et al.*, 2009). The herbaceous crops might not be as beneficial as food crops in agroforestry, but it can be applied as cover crops for supporting the other plants (Bodner *et al.*, 2007; Snapp *et al.*, 2005). We believe that multispecies and multifunctional approach can be advantageous in the four returns restoration projects in at least a timeline of 20 years (Ferwerda, 2016), which integrate the international principles and standards (Gann *et al.*, 2019).

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