Chapter

Essential Role of Symbiotic Microorganisms Supporting Forests in East Asia under Changing Environment

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Abstract

Regeneration success of forests is strongly dependent on symbiotic microorganisms, that is, arbuscular mycorrhiza (AM) and ectomycorrhiza (ECM). In the northeastern part of Asia, larch and birch are used as timber resources, and in the south, fir, cedar, cypress, and oak are used as timber resources. Planted forests have reached the time of harvesting and/or thinning, and after the forestry practices, it is expected that they will become mixed forests equipped with resistance to weather damage; that is, drought, heat, typhoons, etc. On the other hand, the physical production environment has changed greatly, therefore, we investigated the growth of the major trees and the role of mycorrhizal fungi in the northeastern Asia. Elevated O_3 decreased growth, colonization rates of ECM, and the biodiversity; however, elevated CO_2 moderated or increased them in larch. Except for disease of rot and damping off, we discuss wise use of symbiotic microbe in far East Asia.

Keywords: symbiotic microorganisms, conifers, regeneration, mixed forests, changing environment

1. Introduction

Various microorganisms grow in the soil [1, 2]. They are divided into parasitic fungi and symbiotic fungi that attach to living organisms, and saprophytic fungi that decompose and utilize dead things, based on how they acquire nutrients (e.g., [3, 4]). Most land plants form symbiotic associations with mycorrhizal fungi. These below-ground fungi act an essential role in terrestrial ecosystems as they regulate nutrient and carbon cycles [1]. They influence soil structure and ecosystem multifunctionality. Up to 80% of plant N and P is provided by mycorrhizal fungi and many plant species are depending on these symbionts for growth and survival under changing environments [5, 6].

Man-made forests in Japan are mainly composed of coniferous trees and are approaching the final harvest, but the production cost is very expensive and they are still in a state where they cannot be used. After harvesting, mixed reforestation is recommended considering the resistance to various damages [7–9]. On the other hand, since 1999, China has planted *Larix gmelinii* (Dahurian larch) in the north, *Populus* sp. (poplar) in the center, and *Cunninghamia lanceolata* (Chinese fir) [10, 11] in the south as forest rehabilitation as "Gain for Green Program". Recently planted area is the largest in the world, larger than the land area of Japan [12]. Also, in the southwest, *Eucalyptus* sp. (eucalyptus species) are mainly planted and harvested with a method of short rotation intensive culture of about less than 10 years to support the production of wood materials. However, in recent years, there have been cases of concern about the growth of third-generation afforestation areas [13].

Regeneration after wildfires, which frequently occur in the Russian Far East, is mainly carried out by natural regeneration of Dahurian larch, red pine (*Pinus sylvestris* var. *mongolica*), and birch (*Betula platyphylla*) [6, 14, 15]. In both cases, the importance of the role of ectomycorrhizal fungi and biochar has been pointed out (e.g., [16, 17]).

In this chapter, we reviewed briefly on basic information about AM (arbuscular mycorrhiza = vesicular and arbuscular mycorrhiza: VA) and ECM, essential role of symbionts in natural forests [18, 19], Sugi-cedar (*Cryptomeria japonica*: AM conifer) plantation in Japan [9], and regeneration success after forestry practices in northeast China under changing environment [5, 6].

2. Principal of plants and microbe interactions

Four major mycorrhizal types have been described based on their structure and function, namely arbuscular mycorrhiza (AM; formerly named as VA), ECM, orchid mycorrhiza, and ericoid mycorrhiza [18]. In addition, Fabaceae or Leguminosae trees (*Robinia* sp. [20]) and alder tree species (*Alnus* sp. [21]) are also introduced for restoration of degraded lands, especially under nutrient-poor conditions. Therefore, we discuss on effects of nitrogen and ECM colonization (e.g., [22, 23]). Mycorrhizal fungi are a type of fungi colonizing plant roots to form "mycorrhizae" and form symbiosis relations as "mycorrhizal fungi." In contrast to ECM, those whose hyphae enter the cell wall are collectively called "endomycorrhiza."

2.1 General information on AM, ECM, and Rhizobium

2.1.1 Evolution of AM and ECM

Fossil records and molecular clock dating suggest that the ancestors of the plants that entered the land were pteridophytes that appeared 400–300 million years ago and coexisted with AM [24–26] (**Figure 1**). At that time, the CO_2 concentration was about 20 times higher than today, and green plants were photosynthesizing.

Since lignin-decomposing fungi did not exist 400 to 300 million years ago, fossil fuels such as coal and petroleum were produced and accumulated, and then we are using them today (e.g., [27]). About 300 million years ago, gymnosperm (conifers) took precedence, ectomycorrhizal fungi established a symbiotic relationship, and about 150 million years ago angiosperms appeared, and the activities of symbiotic fungi are essential for more than 80% of plants [2, 26, 28].

ECM (about 200 million years ago) and ericoid mycorrhizas (about 100 million years ago) evolved subsequently as the organic matter content of some ancient soils increased and sclerophyllous vegetation arose as a response to nutrient-poor soils, respectively. This section discussed the function of ECM associated with reforestation

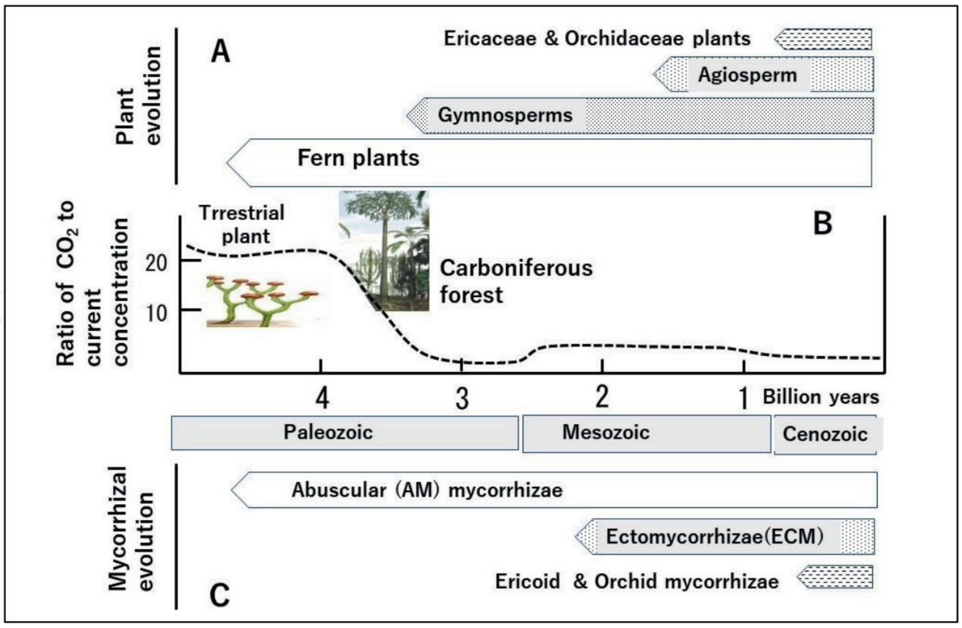

Figure 1.
Evolution of plant (A), atmospheric CO_2 concentration (B), and mycorrhizae (C) (Original figure was offered by M. Saito).

in Sugi-cedar (*Cryptomeria japonica*) and larch (*Larix* sp.) plantations under changing atmospheric environments.

2.1.2 AM

AM fungi have existed since ancient times when plants evolved from the sea to the land and took root on the ground (**Figure 1**). Colonization of plants with AM fungi does not result in significant changes in root external morphology. Inside the root, a unique structure with finely branched tips called "arbuscule" is formed. Fungi whose hyphae form highly branched (arbre: French; tree-shaped) growths (arbuscles) between the cell walls and cell membranes of plant root cells, thereby conveying phosphorous (P) and other materials to plants and taking up organic compounds from root (**Figure 2A2**). Mutualistic symbiotic relationships are maintained by the association of plants. Spores that are present in soil germinate, "infect" the root system, and form arbuscule structures inside the cells of root.

When AM fungi colonize the roots of plants, they absorb phosphoric acid from the hyphae spread in the soil and supply it to the symbiotic plants (hosts) through the dendrites. On the other hand, by receiving energy (carbohydrates) produced by photosynthesis, plants mutually benefit and symbiotically with AM, ECM, etc. [31]. There is a problem to overcome when using AM fungi, which are obligate symbiotic microorganisms, as an inoculation material.

2.1.3 ECM

Ectomycorrhiza (ECM) is a type of mycorrhiza that has hyphae on the surface of the root, mainly fine root and between epidermal cells and cortical cells, but does not enter the cells. The hyphae of ECM usually form a mesh-like structure that is stretched between the intercellular spaces of cortical cells, and this "mesh" is called the Hartig net (**Figure 2A3**, and **B**). In addition, the hyphae form a thick mycelial

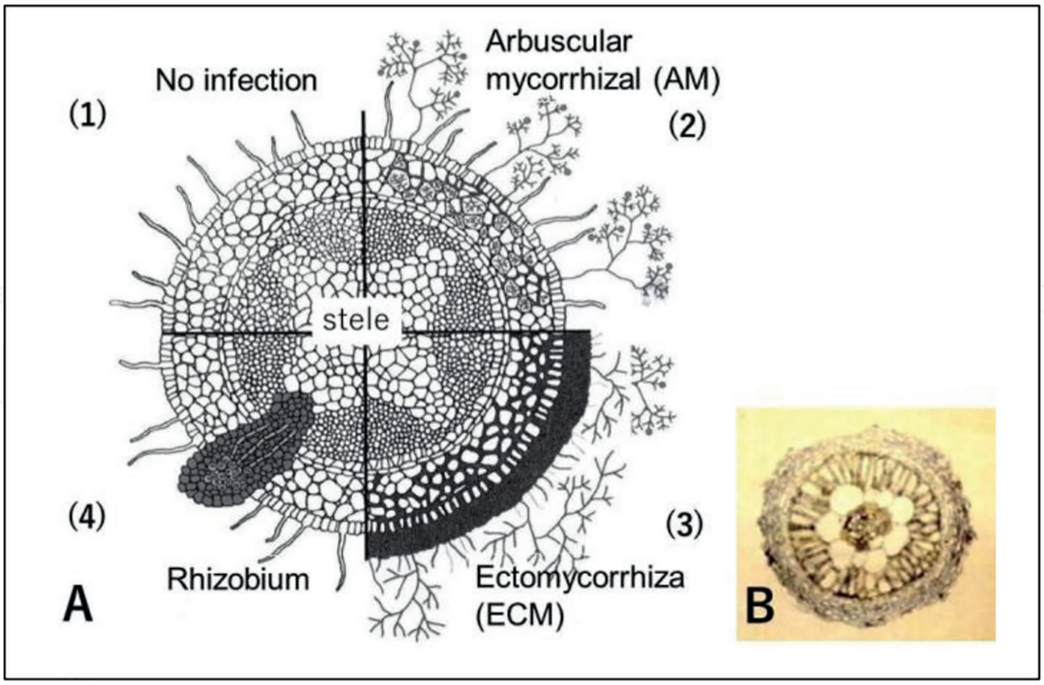

Figure 2.
Illustration of typical mycorrhizae in root (A) and Hartig net (B). A: Modification from Bücking H et al. [29], and B: Courtesy of Y. Matsuda [30].

layer called a fungal sheath (fungal mantle) around the rootlets. The presence of ECM increases the uptake of P, water, and nitrogen (N) by the roots, especially when the concentration of these nutrients in the soil is low [2, 18, 32]. The presence of ECM also contributes to the improvement of resistance to soil pathogens, and the presence of mycelium prevents invasion of other microorganisms and harmful substances.

2.1.4 Rhizobium

Rhizobium is a soil microorganism that performs so-called symbiotic N fixation, forming nodules on the roots of leguminous plants, in which atmospheric N is reduced by nitrogenase, converted to ammonia nitrogen, and supplied to the host (**Figure 2A4**). A symbiotic relationship is established within the nodule by supplying photosynthetic products from the host plant.

Frankia sp. is a genus of bacteria classified as actinomycetes. They have the ability to fix N and live symbiotically with a group of angiosperms called "actinorhizal plants," typically alder (*Alnus* sp.) trees (e.g., [21]). *Frankia* sp. live symbiotically in nodules, which form in the roots of actinorhizal plants. In the root nodule, *Frankia* sp. reduces N molecules to ammonia by N fixation and supplies it to the host plant.

2.2 Nitrogen environment

The activities of symbiotic fungi are enhanced in nutrient-poor environments (e.g., [18, 33]). Therefore, increased N deposition reduces the health and vitality of symbiotic forest ecosystems. N is usually insufficient n temperate and boreal forests [34], and according to Aber et al. [35], who first noted the effects of N deposition as four stages, growth is promoted in the initial stage (stages 0 and I). However, N addition experiments on nutrient-poor soil in North America began to decline (stages

I and II), and excess N flowed into the watershed (stage III). The activity of mycorrhizal fungi can be expected at Stage I or below.

In general, forests circulate nutrients through litterfall as a self-fertilizing function (e.g., [36]). Deciduous forests in Japan tended to be 10–30 kgN hr^{-1} yr^{-1} higher than coniferous forests in values before air pollution became serious in the 1960s. Therefore, in places where N deposition exceeds the amount of self-fertilization, attention must be paid to N runoff. In an experiment using tree seedlings [37], nitrogen (N) fertilizer was added up to about 80 kgN hr^{-1} yr^{-1} and found the N tolerance. in the order of *Larix gmelinii* x *kaempferi* (Hybrid F_1), *Cryptomeria japonica* (Sugi-cedar), *Abies firma* (Fir), *Picea asperata* (dragon spruce), and *Quercus acuta*. However, growth of following tree seedlings is suppressed with 50 ~ 80 kg N hr^{-1} yr^{-1}; *Pinus densiflora* (red pine), *P. tabutaeformls*, *Fagus crenata* (Siebold's beech), *Castanopsis sieboldii,* and *Q. glauca* (evergreen oak).

3. Essential role of symbiotic microbes in a mixed forest

Many man-made coniferous forests in Japan are approaching the harvest, but the cost of harvesting and transporting the timber is too expensive to make use of it and price of raw material (=timber) is too low [38, 39]. Tending the plantation is essential because delaying the harvesting period will result in strong wind, heavy snowfall, etc. Although there is an administrative guideline to delay the harvest time, if implemented, weather damage like typhoon will occur, therefore, forest tending is essential for conservation of land and biodiversity (**Figure 3**) [40].

A framework is proposed for considering how tree species and their mycorrhizal associates differentially couple carbon (C) and nutrient (mainly N) cycles in temperate forests [41]. In AM-dominated forest stands (**Figure 4A**), an inorganic nutrient economy is predicted as a result of the fast decomposition of high-quality litterfall (=high nitrogen content with low defense chemicals, such as phenolics including lignin) and enhanced mineralization rates of C and nitrogen (N) [42]. In contrast, an organic nutrient dynamic is estimated as a result of low-quality litterfall (=low N with high phenolics) and slow turnover rates of C and N in ECM-dominated stands (**Figure 4B**). These trends may indicate limited losses of inorganic nutrients.

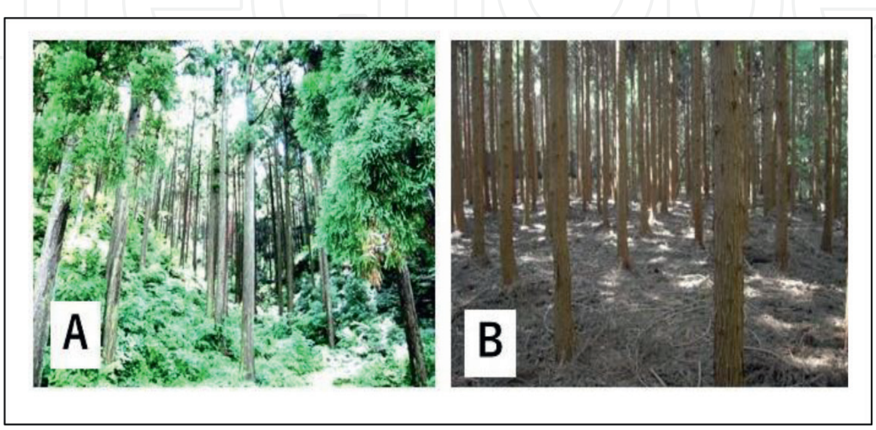

Figure 3.
Sugi-cedar (Cryptomeria japonica) plantations with adequate thinning (A) and no thinning (B) in Southwestern Japan (after [40]). Forest floor is an aerobic condition with rich vegetation (A) vs. that an anaerobic condition with no ground vegetation (B). Photos are courtesy of E. Takahashi.

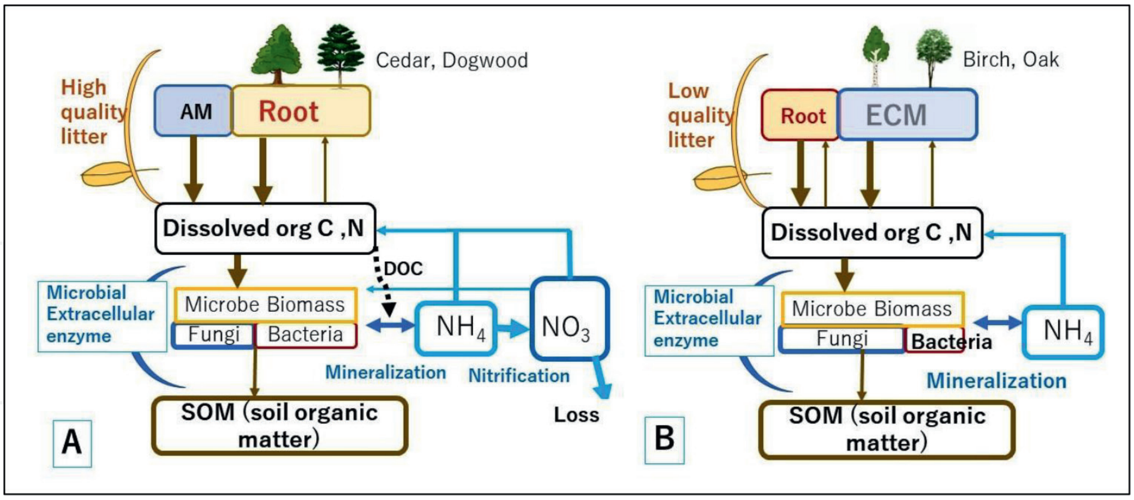

Figure 4.
Trees predominantly associate with a single type of mycorrhizal fungi AM or ECM, and the two types of fungi differ in their nutrient acquisition; abundance of AM (A) and ECM (B) trees may provide an integrated index of biogeochemical transformations relevant to C cycling and nutrient retention in a forest ecosystem. Within a mycorrhizal group, the size of the boxes and arrows indicates relative importance of pools and fluxes, respectively (Modification from Phillips et al. [41]).

In order to improve soil fertility and avoid weather damage, it is necessary to promote mixing with broad-leaved trees for biodiversity conservation [9]. This statement is applicable to the large-scale plantations in China and in South Korea. This section discussed the case of Sugi-cedar (AM conifer) plantation in the cool temperate zone in Japan for making a mixed forest and moderating methane (CH_4) emission in closed forests [43].

3.1 Conifer plantation to mixed broad-leaved forest by thinning

Thinning is an effective method to create mixed broad-leaved trees (hardwoods)—conifer forests with successful hardwood recruitment [44]. However, it remains unclear to what extent mycorrhizal association (AM vs. ECM) affects hardwood recruitment. In this section, we explored which mycorrhizal types of hardwood were more advantageous to recruit into a Sugi-cedar plantation in northern Japan [9, 45, 46].

We compared seedling recruitment success (i.e., seedling emergence, survival, and growth) between AM and ECM hardwoods during 5 years after thinning at two levels: 67% thinned (intensive), 33% thinned (weak), and un-thinned treatments (control). We also evaluated the colonization percent of mycorrhizal fungi for the seedlings of the most dominant AM (basswood: *Cornus controversa*) and ECM (*Quercus serrata*) hardwoods in both the Sugi-cedar plantation and adjacent forests dominated by ECM tree species (e.g., *Q. serrata*, *Alnus hirsuta* var. *sibirica*, *Castanea crenata*) [30, 45–48].

From 5 years of monitoring, rate of emergence and survival of seedlings was greater in AM-type broad-leaved tree species than in ECM-ones. Height growth of the seedlings was also greater for AM than ECM species, especially for early emerging cohorts in thinning treatments [45]. Colonization rate of AM fungi to AM seedlings was greater in the Sugi-cedar (AM-type) plantation than in the adjacent ECM-dominant forest stands. Conversely, colonization rate of ECM fungi to ECM-type seedlings was lower in the plantation than in broad-leaved forests. These results suggest that AM broad-leaved trees could easily be recruited to AM conifer plantations.

Growth of the AM seedlings was enhanced, which translocates nutrients by way of hyphal networks [18]. Recruitment of ECM-type seedlings was strongly inhibited; however, this may be due to the lack of association of the ECM fungi in the AM-type conifer (Sugi cedar) plantation. Consequently, mycorrhizal associations play an important role in recruitment success of mycorrhizal broad-leaved trees into AM conifer plantations.

3.2 Methane emission and the possible role of earthworms after thinning

Methane (CH_4) is the second strongest greenhouse gas after CO_2, excluding water vapor, and forests currently act as a source of CH_4 consumption (= CH_4 absorption; e.g., [34, 49]).

However, due to the increase in atmospheric CO_2 concentration, foliar density of canopy trees increases larger, and the incident light to the forest floor decreases (e.g., [50–52]), inducing the forest floor in a moist state (= anaerobic condition) [53, 54]. Furthermore, looking at individual leaves, stomata tend to close with increasing CO_2, so transpiration is suppressed [51, 52]. Therefore, the water content of the soil, in which the root system develops, is maintained at a high level. In the case of brown forest soil (Cambisols), CH_4 is released when the atmospheric CO_2 concentration is about 500 ppm and the soil moisture content exceeds 35% (**Figure 5A**) [55].

However, although thinning reduces the transpiration surface of the canopy, the rise in soil temperature promotes the activity of earthworms, and the earthworms eat fallen leaves or litterfall that cover the ground surface andprevent evaporation. These earthworm activities promote drying of soil surface on a forest floor (**Figure 6**) [56]. Increased soil porosity and near-surface aerobic conditions by earthworms [9, 56] can keep the methane-consuming capacity of forests. These tendencies were confirmed in AM-type cedar forests (**Figure 5B**) [43]. ECM-type mixed spruce-broad-leaved forests [56] and ECM-type birch-oak-beech stand with a free-air CO_2 enrichment (FACE) [49].

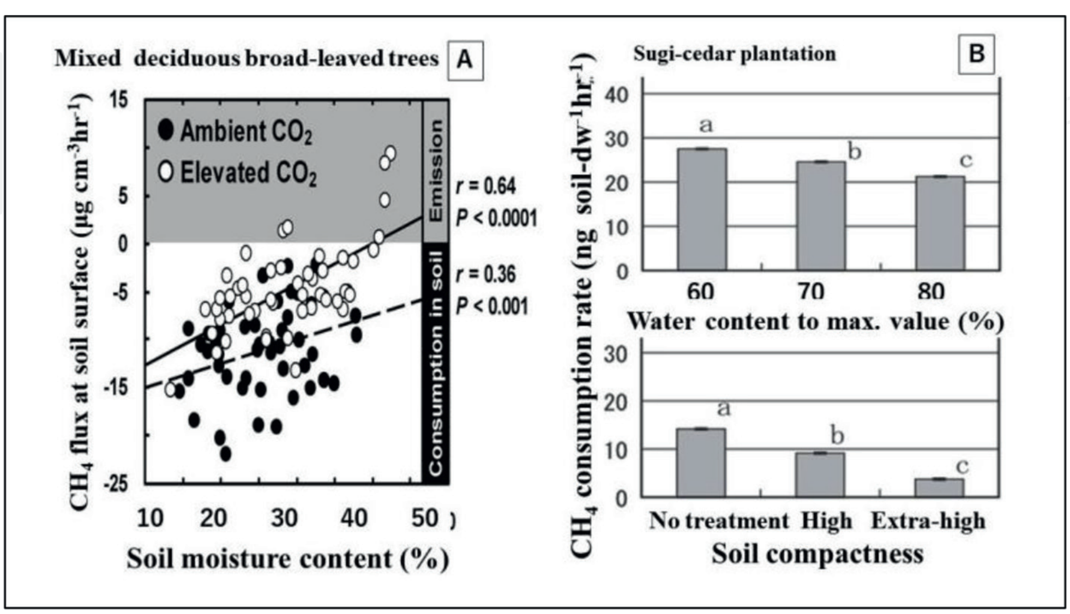

Figure 5.
Methane consumption rate under various conditions. Adopted from (A) Kim et al. [44, 50], (B) from Mametani et al. In B, different letters indicate significant differences ($p < 0.05$), [40].

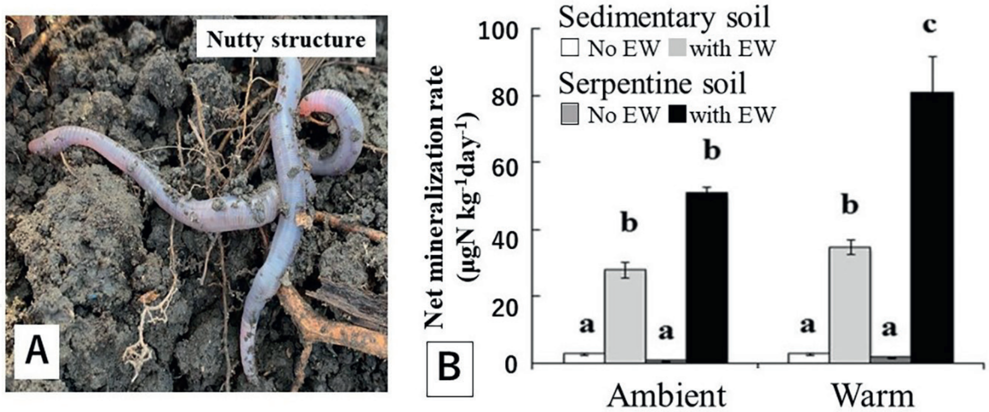

Figure 6.
Nutty soil structure by earthworm (A) and net mineralization without/with Earthworm in different soil fertility (rich-sedimentary soil; poor-serpentine soil under elevated Temperature (+3°C) (B). A: Courtesy of Makoto K, B: adopted from Makoto et al. In B, different letters indicate significant differences ($p < 0.05$), [45].

3.3 Reforestation at acidic and infertile sites with mycorrhizae

Seedlings of three kinds of evergreen *Fagaceae* species (*Quercus glauca, Q. salicina, Castanopsis cuspidate*) planted on acidic [57], nutrient-poor colluvial soil showed accelerated growth when inoculated with ECM fungi [58, 59]. Of course, differences in growth among the seedlings seem to be strongly related to differences in nutrient acquisition of species. Utility of ECM (*Astraeus hygrometricus* or *Scleroderma citrinum*) inoculation to the tree seedlings was examined for their growth in acidic soil conditions with no information on native bacterial flora of ECM. Six months after planting, seedlings of the three species inoculated with *A. hygrometricus* were growing well, especially, *Q. salicina* (**Figure 7**). The growth of seedlings inoculated with *S. citrinum* was inferior to seedlings inoculated with *A. hygrometricus*.

This difference in inoculation effect of two ECM species to *Q. salicina* seedlings may be attributed to an affinity between host-symbiont. Acidic soils are widespread in many parts of the world, turning them into wastelands. Phosphorus (P) is adsorbed on the soil and cannot be used by plants [18]. P is available to host plants through organic acids produced by AM and ECM. Helper bacteria perform important activities when the holobiont is formed [60, 61]. Furthermore, when soil pH < 4.5, toxic Al is liberated and inhibits root system function. However, some *Melastoma* sp. growing in tropical peatlands accumulate Al in their roots to prevent excessive accumulation of Fe, which inhibits growth in acidic environments [62].

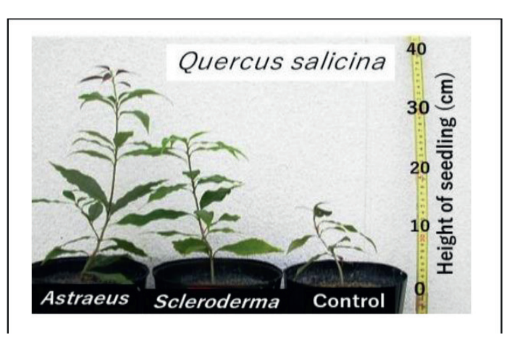

Figure 7.
Growth of an evergreen oak inoculated with different ECM (Astraeus hygrometricus vs. Scleroderma cepa) vs. no-ECM inoculation after 4 months in a phytotron (by courtesy of M. Kayama).

4. An application of symbiotic microbes in regeneration of the man-made forests

4.1 Principal idea of mycorrhizal technology

With the implementation of China's key forestry projects such as Natural Forest Conservation Program (NFCP) and conversion of farmlands with steep slopes to man-made forests, the area of zonal secondary broad-leaved forest has increased rapidly in subtropical areas [11]. Among them, afforestation projects are carried out on barren mountains, wastelands, and degraded lands. In order to improve the ability of trees to survive and grow under natural harsh conditions, attention should be paid to wise use of the symbiotic relationship between trees and ECM and AM, which is the key to the success of afforestation.

Mycorrhizal colonization with the seedlings has become the third factor of afforestation after "matching species with the site, improved variety, and strong seedling" (e.g., [58, 63]). China has been committed to the application and promotion of mycorrhizal technology. Namely, the Chinese Academy of Forestry led the implementation of forest mycorrhizal biotechnology project, which has played an important role in improving the quality and the survival rate of seedlings in afforestation.

4.2 Role of biochar in afforestation and regeneration after forest fire

Forest fires occur frequently in the Far East, especially on the Russian territory, where large areas are burning, but regeneration takes place relatively quickly outside the salt accumulation areas [14, 64, 65]. The burned forest floor is full of charcoal with few surviving individuals (**Figure 8**). If the thick-barked larch (*Larix gmelinii*) trees would not receive a canopy fire, the surviving individuals become mother trees to regenerate naturally. Experimentally, charcoal is effective for the growth of seedlings of larch and pine, in which it is located about 1 cm above the ground surface but not randomly distributed [66] and the charcoal combustion temperature is relatively low (<400~600°C) [17, 67].

As charcoal is a carbon-enriched material with a highly aromatic and porous structure, it is highly resistant to microbial decomposition and thus remains in soil for thousands of years. The abundant pores existing charcoal surfaces have powerful adsorption.

Figure 8.
Forest regeneration after forest fire in the Russian Far East (by courtesy of K. Makoto for A and C; of Y. Matsuura for B) (A) After stem fire, (B) Survived larch individuals with thick bark and regenerated seedlings, and (C) Larch seedlings with biochar.

Charcoal on soil-plant systems explains the complex direct and indirect pathways of these influences that occur during succession after fires in boreal ecosystems. It influences soil-plant systems related to the element composition and nutrient availability in soils [16, 68] and to the abundance of phenolics released from *Ericaceae* plants in the understory of boreal forests [69]. We found a strong bias in the studied processes toward nutrient mineralization rather than immobilization, which suggests that it is risky to draw general conclusions about the influence of charcoal on soil nutrient dynamics. Artificial forest fire experiments gave information about an increase incident light to forest floor and enhancement of litter and humus decomposition and moreover, given the possibility that charcoal accelerates CO_2 release in a postfire forest for relatively long-term [67].

4.3 Afforestation using seedlings associated with ECM

Some conifer species (e.g., pine) and broad-leaved tree species (e.g., oak) are tightly dependent on ECM fungi in their natural state (**Figure 7**), otherwise, they cannot survive [70]. In the early years, China had repeatedly failed to introduce foreign pines because of the lack of symbiotic mycorrhizal fungi, which made the introduced plants grow poorly or even unable to survive until the mycorrhizal fungi of origin of pines are introduced. Commercial ECM fungi: *Pisolithus tinctorius* and *Scleroderma polyrhizum* use practically for "greening material" in pine species and larch [63, 71] and eucalyptus and acacia (e.g., [72]), which can promote the growth of host plants and improve the stress resistance of host plants. At initial stage, ECM is hardly inoculated with host plants because ECM requires lots of photosynthates, therefore, we should introduce "helper" bacteria for accelerating symbiotic relations [73, 74].

After ECM inoculation, the seedlings had a positive effect on seedling height, ground diameter, total biomass, and root diameter. This was found in the studies on *Pinus tabulaeformis*, *Cunninghamia lanceolate*, *Taxus cuspidate,* and *Cyclobalanopsis gilva* [75]. The inoculation of ECM fungi can improve the quality of container larch F_1 seedlings [76, 77] and reduce the use of slow-release fertilizer, which is helpful to cultivate high-quality seedlings and save seedling costs [78] but not always true for larch seedlings [71, 79, 80].

AM fungi also play an important role in maintaining the stability of the southern coastal mangrove ecosystem. Plants in the mangrove ecosystem can form a symbiotic system with a variety of AM fungi under aerobic conditions. Inoculation with AM fungi can significantly increase plant height, stem diameter, and aboveground biomass of mangrove apples (*Sonneratia caseolaris*) and promote the uptake of phosphorus and potassium by roots [81]. Yuan et al. [82] found that AM fungi could promote the uptake and utilization of potassium nitrate and ammonium sulfate in NO_3-N fertilizer by oil trees (*Elaeagnus mollis*) seedlings. In the experiment of inoculation AM fungi and rhizobia on shrub legume (*Amorpha fruticosa*) found that there is an interactive recognition process between mycorrhizal fungi and rhizobia, that double inoculation could significantly increase the mycorrhizal colonization rate and nodule number [83–85].

The rate of mycorrhizal colonization and the number of nodules by double inoculation were significantly higher than those by other inoculation treatments [63, 71, 86, 87], which enhanced the nitrogen-fixing ability of rhizobia and promoted plant growth.

4.4 Afforestation in adversity and biological control

Mycorrhizal colonization of the plants (=**holobiont**; e.g., [88]) can improve both the survival rate of seedlings and the absorption and utilization of nutrients in soil by holobiont. A holobiont is defined as an assemblage of a host (plant) and the many other species living in or around it. Once holobiont is formed, symbionts promote the growth of host plants, and we expect an enhancement of the resistance of seedlings to diseases, drought, organic pollutants, and heavy metal stress [89]. In order to improve the survival rate of afforestation tree seedlings, researchers suggest using the mutualistic symbiotic relationship between mycorrhizal fungi and host trees to increase the success rate of afforestation under adverse conditions in non-forested areas and degraded lands where soil and vegetation have been destroyed (e.g., [30, 33, 63, 88, 90]).

Inoculation of ECM fungi in acidified soil in southeast Sichuan, China, could improve the viability and aluminum (Al) resistance [62, 91] of *Pinus massoniana* in poor soil, which increases the local coverage [92]. Under the conditions of copper (Cu) and cadmium (Cd) stress in soil, the inoculation of ECM fungi on *Pinus tabulaeformis* can promote not only the growth and development of host plants and increase the biomass but also significantly reduce the concentration of heavy metals. ECM-host plants (holobiont) moderate the transfer of heavy metals from roots to shoots, improved the resistance to heavy metal stress, and improved the survival rate of forestation [88, 93].

In the process of coal gangue hill reclamation, inoculated ash tree (*Fraxinus chinensis*) seedlings with AM fungi, survival rate of the transplanted seedlings was significantly improved [42, 94]. They also found that ECM (*Gomphidium viscidus*) can significantly alleviate effect of coal gangue on the growth of *Pinus bungeana* seedlings, and the number of lateral roots and dry weight of inoculated seedlings were significantly higher than those of uninoculated seedlings, so as to promote the ecological restoration of gangue areas.

Using mycorrhizal fungi for biological control of tree diseases has the advantages of low cost, high efficiency, avoiding environmental pollution caused by chemical control, and can promote the robust growth of seedlings. The ECM fungi (*Boletus edulis*, *Amanita pantherine, Pisolithus tinctorius*) could enhance the resistance of *Pinus massoniana* seedlings to pine dieback disease, and also enhance the resistance of pine (*P. thunbergii* and *P. massoniana*) to pine wood nematode disease, delaying or reducing the death of seedlings [95]. Meng et al. [96] inoculated ECM against the damping-off of Dahurian larch (*Larix gmelinii*) seedlings in the alpine mountains and found that, compared with the control area, the incidence of damping-off decreased by 14–22%, and that of European red pine (*P. sylvestris*) decreased by 12–20%.

4.5 Edible mushroom production

ECM has always been a keyword with high frequency in the field of mycorrhizal research in China, mainly because the symbiotic relationship between ECM fungi and gymnosperms has great guiding significance for afforestation [97]. In addition, the fruit bodies of various ECM fungi are edible mushrooms with high economic value [98]. With the deepening of people's understanding of the edible or medicinal value of ECM fungi, more and more scholars are committed to the research on the artificial cultivation technology of ECM fungi. China has rich in wild edible fungi resources [99]. At present, the production of ECM edible fungi abroad has formed a certain scale in China, which has played a significant role in the national economy and brought great wealth.

From the holobiont viewpoint, an increase in host plant photosynthesis by thinning may allocate more photosynthates to belowground associated with ECM, and then increase mushroom production [100, 101].

5. Regeneration of seedlings on fallen logs

In forests, there are symbiotic fungi that play an important role in the acquisition of nutrients and water after seedlings have settled. However, it has long been known that AM, ECM and some kinds of fungi damage and cause seeds to rot and damp off in seedlings [102, 103]. Here, we discuss the role of CWD in the regeneration of natural regeneration. At this time, we introduce the activity of fungi that decays the CWD, which acts as a seed and seedling bed and helps naturally regenerate the forest.

5.1 Role of CWD

Regeneration of spruce (*Picea* sp.) in high-elevation mountain or cool temperate forests often depends on the presence of coarse woody debris (CWD), as logs provide sites with more favorable conditions for spruce regeneration compared to the forest floor (**Figure 9**) [104, 105]. However, there is little quantitative knowledge on the factors that are conducive to or hindering spruce establishment on CWD.

Variables describing microsite conditions were measured, and fungi were isolated from wood samples. Seedling density depended on the type of rot, log inclination, and decay stage of logs. Sapling density depended mainly on light availability, the time of tree fall, and the distance between the log surface and the forest floor [105]. A total of 22 polypore fungi were isolated from the wood samples, four of them being threatened species as disease sources [104]. White- and brown-rot fungi were found in all decay stages. The visual assessment of the type of rot in the field corresponded in only 15% of cases to the wood rot type caused by the isolated fungi; we should pay attention to the presence of harmful microbes and make field assessments of rot type [99].

5.2 White rot vs. brown rot

Seedling and sapling densities followed negative binomial distributions, with many logs of all decay stages having low regeneration densities [105]. The degree of ground contact, white-rot-causing *Armillaria* spp. presence, white-rot-causing *Phellinus nigrolimitatus* presence, and log diameter were positively related to both seedling and sapling density [99]. Also, tree death as a result of wind uprooting was positively related to seedling density. Conversely, the presence of brown-rot-causing

Figure 9.
Natural regeneration from spruce seedlings on fallen trees with brown rot in temperate mixed conifer-deciduous broad-leaved forests in northern Japan.

Fomitopsis pinicola and tree death as a result of bark beetle attack was negatively related to regeneration densities. The low cover of vegetation from sides positively affected seedling density; however, heavily covered logs were less occupied by seedlings. Former studies provide evidence that large logs originating from wind uprooting or butt rot infection are most appropriate [104, 106].

6. Function of symbiotic microbes under changing environment

We have discussed the role of symbiotic fungi in forest regeneration and tending practices, therefore, we need to further discuss forest management that responds to the activities of symbiotic fungi in response to rapid environmental changes, including recent global warming by increasing CO_2 [90, 107, 108]. As most of them are experimental studies [22, 109], we will introduce examples of rehabilitation in acidic soils and salinized soils as well as physical environmental changes (e.g., elevated CO_2, ozoneO_3, N loading).

6.1 CO_2, O_3, and N loading

Several Pinaceae species (*Pinus* sp. and *Larix* sp.) in northeastern Asia, especially Korea, show declining symptoms due to soil acidification and air pollution [63]. In the industrial area of northwest Korea, growth and physiological activities of *Pinus koraiensis* and *P. rigida* are adversely influenced by increased deposition of fluorine (F), chlorine (Cl), and manganese (Mn), involving both wet and dry deposition; these also have harmful effects on the ectomycorrhizal status [110]. The ECM colonization level at the damaged site was 30% less than at a control site. To determine the role of ECM with seedlings of *P. densiflora, P. koraiensis, P. rigida,* and *Larix kaempferi* during progressive soil acidification, the relation between relative total dry mass (TDM) and the molar ratio of base cation (BC) to aluminum (Al) (BC/Al) BC to manganese (Mn) or BC/(Al + Mn)) was examined [63].

The TDM of *P. koraiensis* and *L. kaempferi* seedlings was approximately 40% at a BC/Al ratio of 1.0, but that of *P. rigida* and *P. densiflora* was approximately 50% at the same BC/Al ratio. With an increasing amount of proton (H^+) added to the soil, the water-soluble concentrations of Ca, Mg, K, Al, and Mn increased, especially below a soil pH of 3.8. Development of ectomycorrhizae, that is, *Pisolithus tinctorius* (*Pt*), Ectodrench (EC), or *Cenococcum geophilum* (*Cg*) in all four test species was significantly greater in the 10 and 30 mmol$H^+ \cdot kg^{-1}$ treatments than in control treatments [63]. ECM development in the 60 and 90 mmol$H^+ \cdot kg^{-1}$ treatments was significantly lower than in the other treatments. Atmospheric CO_2 increases yearly, which may affect mycorrhizal growth and diversity in forest ecosystems through host plant physiology.

The ECM development of each species was significantly higher under elevated CO_2 concentration (720 ppm) than at ambient levels (360 ppm) in the study at the time [63]. The P concentration in needles and roots following inoculation with *Pt,* EC, or *Cg* was significantly higher in all species than without inoculation, at both CO_2 concentrations. Furthermore, no photosynthetic down-regulation was found in any species when inoculated with *Pt,* EC, or *Cg,* and photosynthesis was significantly higher than in non-inoculated seedlings at both CO_2 concentrations [111]. Stomatal limitation in each species at each CO_2 concentration was less than in non-inoculated seedlings [71].

Simulated nitrogen (N) deposition (50 kg N $hr^{-1}yr^{-1}$) increased photosynthesis and growth of hybrid larch F_1 for the initial 2 years. No significant effects were

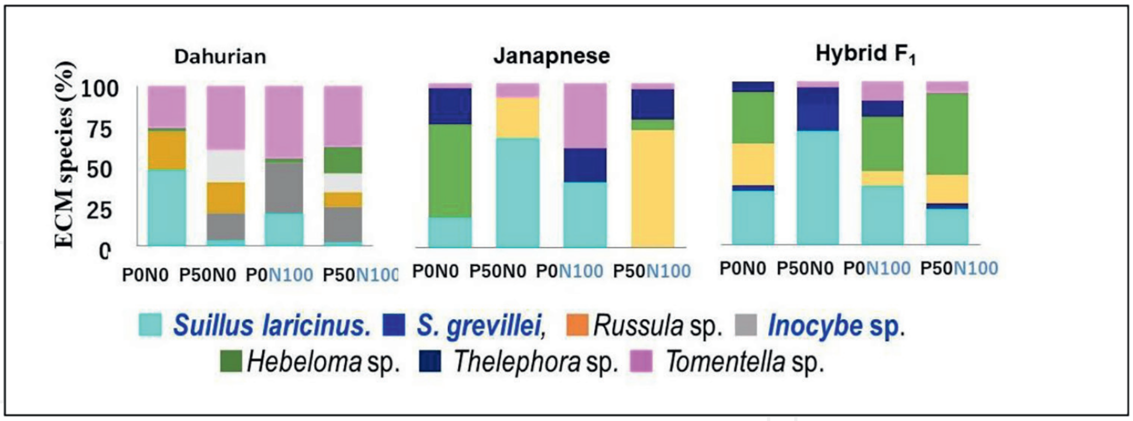

Figure 10.
Ectomycorrhizae (ECM) colonization of three larch species grown under different N and P (as solution of ammonium nitrate (NH_4NO_3) and potassium phosphate (KH_2PO_4)). The P50N100 means 50 kgP ha^{-1} yr^{-1}, 100 kgN ha^{-1} yr^{-1} for four growth seasons, and zero indicates no nutrient treatment. Suillus larcicinus, S. grevillea, and Inocybe sp. are specialist ECM to larch. However, Suillus grevillea and Inocybe sp. were only found in F_1 and Dahurian larch (DL: Larix gemelinii), respectively [23].

found for the next 3–8 years on brown forest soil (Dystric Cambisols). Among three larch species (Japanese, Dahurian, and F_1 hybrid larch), the dominant ECM species preferred N (**Figure 10**) [23].

Elevated O_3 decreased growth of larch seedlings, ECM colonization rates, and ECM species diversity; however, elevated CO_2 moderated or increased them in hybrid larch F_1. After elevated CO_2 and O_3 fumigation on F_1 seedlings, we found *Suillus* sp., a larch specialist ECM in F_1 [112], which means these ECM will be effective materials for larch plantations [97].

6.2 Combination effect of salt stresses

Soil salinization area is estimated to increase due to global warming [113], especially low precipitation region in the northeastern part of Asia. Inoculation of mycorrhizal fungi can often promote the growth of plants in a saline environment, increase the tolerance of plants to excessive sodium (Na) ions in the environment, and reduce the toxicity of mycorrhizal fungi to plants. Mycorrhizal fungi increase the ability of plants to resist salt stress by promoting the absorption of mineral elements, promoting the balance of elements in plants, and improving the water state of plants [95]. In addition, due to rapid development of industrialization and urbanization, emissions of ozone (O_3) precursors have been increasing rapidly, which has led to pronounced air pollution of ground-level O_3 [5, 114]. The effects of elevated ozone (eO_3) and soil salinization with alkaline salts in northeastern (NE) China is a serious concern affecting the success of the national replanting project [115]. As planted areas exceed 4 million hectares in China, we must consider future afforestation efforts after thinning and harvesting.

We evaluate the effects of elevated O_3 and alkaline salt on Dahurian larch F_1 (DL: *Larix gmelinii* var. *japonica*) and Japanese larch (JL: *L. kaempferi*) seedlings, and their hybrid larch F_1 (hereafter F_1, *L. gmelinii* var. *japonica* x *L. kaempferi*). As a result, effect of elevated O_3 on three larch species has hardly been found to interact with salinity treatments [116]. However, based on a leaf nutrient analysis, DL and JL have certain negative influences by the salinity treatment. The relationship between needle Na and other mineral contents indicated that both maintained K contents even with excess Na contents in needles. DL showed relatively lower reduction of other mineral contents, indicating higher salt tolerance of needle element homeostasis than JL. Low salinity stress did not significantly inhibit the growth of F_1 and even promoted it to some extent. There were no interaction effects of eO_3 and salt stress on both species. These results indicated that DL seedlings may be more suitable than JL seedlings as a future afforestation species under eO_3 levels of about <70 ppb at saline soil conditions [115].

In northern Japan, the growth of *Picea abies* and *P. glehnii*, which have been planted along the highways, is often suppressed due to several environmental stresses [89]. To examine the adverse effects of deicing salt (mainly NaCl), we measured ECM colonization, needle lifespan, and physiological capacity of the two spruce species at a site with damaged trees, near the roadside, and a site with healthy trees. These elements (Na and Cl) accumulated in spruce needles from the soil and snow at the damaged site. However, no ECM colonization was found in both spruce on the damaged site. Poor physiological traits might be attributed to an accumulation of deicing salt in the needles, resulting in the suppression of tree growth.

7. Conclusion

Fungi, which have coexisted with land plants since ancient times, play an important role in regenerating forests and maintaining and improving ecosystem services. In high CO_2 environments, commensal fungi act as sinks and play an important role in maintaining ecosystems. Methane (CH_4) is the second largest greenhouse gas after CO_2. At present, forests are methane sinks, but as the CO_2 concentration increases, the leaves in the upper canopy grow thicker, suppressing transpiration and creating an anaerobic state, turning them into sources of methane emissions. To avoid this, and maintain forests as a source of methane consumption, it is necessary to keep the forest floor dry (aerobic conditions). In addition, since monoculture forests are not resistant to various damages; therefore, mixed forests are required. In order to maintain a bright forest floor, thinning should be carried out intensively and direct sunlight should be introduced to raise the soil temperature, and the activity of earthworms would make the forest floor an aerobic condition. For these, we should introduce broad-leaved trees with AM and/or ECM to expect natural regeneration after thinning or harvesting conifer trees. We would like to expect charcoal to enhance the activities of symbiotic fungi that are involved in every aspect of forest regeneration, even in the rapidly changing physical environment.

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