The influence of substrates rates on the germination characteristic of a soil seed bank

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Abstract. Soil seed bank (SSB) is considered as an important way of vegetation restoration, it can fleetly achieved vegetation diversification and the course of succession when the topsoil mixed with planting substrates. In this paper, a greenhouse germination method was used to explore the effect on germination characteristic of soil seed bank by adding different inorganic substrates, such as activated carbon, perlite and vermiculite. The results showed that perlite and vermiculite can effectively promote the germination of soil seed bank, but also significantly promote Shannon-Wiener diversity index. When vermiculite mixed with the topsoil in 40%, the germination effect of soil seed bank was more obviously than other groups ; at this time, the density of soil seed bank reached $6 \times 10^5$ plants/m², Shannon Wiener diversity index reached 1.4354. Therefore, it was more conducive to improve the soil seed bank density and species diversity by adding 40% vermiculite in the topsoil.

1. Introduction
Soil seed bank (SSB) is a collection of viable seeds present in the soil and on its surface with regeneration potential. As the “genetic potential library”[1] in the ground vegetation, soil seed bank plays an extremely important role in the process of vegetation restoration and vegetation potential updates[2]. In recent years, with the international status of the degraded damaged land and ecological system's restoration and reconstruction rising, the research of vegetation degradation's soil seed bank were studied extensively and deeply in our country (such as grassland, wetland and abandoned land's degradation [3-5]). However, soil seed bank's research which can be applied to the actual vegetation restoration is still few.

The research of soil seed bank on foreign country started earlier and the application in engineering practice also had a breakthrough, especially there were lots of soil seed bank's application case in Japan, such as using soil seed bank applied to the vegetation restoration for reservoir bare land, waste dump and high-speed road surrounding [6-7]. In addition, the Japanese scholar have researched on spray seeding afforestation methods in topsoil mixed with planting substrates, which mainly reflected in the forest vegetation restoration[8] and slope greening[9]. Based on the above reasons, a greenhouse germination method was used in this study, in order to explore the influence of different rates of inorganic substrates on the germination characteristics of soil seed bank, and provide valuable reference on soil seed bank for the future application in vegetation restoration project.
2. Materials and methods

2.1. Topsoil sampling site
Sampling site was located in Tianjin Wuqing District, China (E116° 43'46"-E117° 19'59", N39° 07'05"-N39° 42' 20"), which belonged to the typical suburban junction area, and also had relatively rich flora and soil seed bank diversity. The matter accumulation quantity of soil organic was not much, and the soil was slightly alkaline. There were abundant plant species, such as *Ulmus pumila*, *Fraxinus velutina*, *Tamarix chinensis*, *Robinia pseudoacacia*, *Morus alba*, et al.

2.2. Sampling method
The research started in March 2014, 30 samples were randomly selected from the sampling site (the sample area was 1 m× 1 m), and collected 0-15 cm of topsoil as a soil sample. The samples were mixed evenly and removed the impurities (sand, stone, leaves, etc.), then arranged samples in snakeskin bag and brought them to the laboratory. Meanwhile, the vegetation condition was recorded, including species composition, quantity and vegetation coverage.

2.3. Experimental design
In the practical seedling applications, such as agriculture, horticulture and flower cultivation, the use of substrate is a very common phenomenon [10-11]. In this study, three kinds of inorganic substrate were selected, in which included activated carbon (A), perlite(P) and vermiculite(V). The reason for choosing these substrates were that these substrates were widely used and easily obtained in Tianjin.

And then, a greenhouse germination method was used to explore the germination characteristic of soil seed bank, in which include the germination density, Shannon-Wiener diversity and the dynamic of seed germination. The germination greenhouse was located at the Tianjin University of Technology, where germination experiments were implemented. After removal of gravel or dead leaves, the soil samples were mixed with three kinds of inorganic substrates in 10%, 20%, 30%, 40% respectively. The mixture of substrate and topsoil were laid in the seedbed to a thickness of 5cm (the size of germination disk is 50 cm×20 cm×5cm). In addition, this research provided a set of blank control group (without adding any substrates). The germination experiment began in May 5th and ended in May 30th. The experiment needed to keep the soil moist, and record the amount and type of soil seed germination and other data regularly.

2.4. Data analysis
The research data were all analyzed and disposed in Excel and R3.0.2 software. In the calculation of the community diversity index, the Shannon-Wiener diversity index was selected in this study, and the formula was as follows:

\[
H = - \sum_{i=1}^{S} (P_i \times \ln P_i)
\]  

In the formula: \(P_i\) was the ratio of germination amount of plant \(i\) to the total germination amount of the soil seed bank.

3. Result and discussion

3.1. The germination density of soil seed bank
As shown in figure 1, relative to the blank control group (the dashed line), perlite and vermiculite effectively promoted the germination of the soil seed bank, but activated carbon had little effect on seed germination. When vermiculite mixed with topsoil in 40%, the density of soil seed bank was obvious, as much as \(6 \times 10^5\) plants/m\(^2\). Due to the inorganic substrates had larger porosity, which could promoting the air circulation and keeping moisture, and it also could promoting the growth of the plant roots and the germination of soil seed bank; in addition, when it compared with the other
inorganic substrates, the fertilizer ability of activated carbon was much worse which caused the little effect on the density of soil seed bank.

Figure 1. The germination density of soil seed bank by adding different inorganic substrates (units: $10^5$ plants/m$^2$).

3.2. Shannon-Wiener diversity index
As shown in figure 2, relative to the blank control group (the dashed line), most of the groups improved the Shannon-Wiener diversity of germination species, especially perlite and vermiculite had significant promoting effect on Shannon-Wiener diversity index, while activated carbon in 10% to 30% has inhibitory effect on it. Through analyzed the type and the quantity of seed germination species, found that adding 10%-30% activated carbon in topsoil greatly promoted *Setaria viridis* growing (more than 80% of the total number of seedlings germination). Shannon-Wiener diversity index not only related to the species, but also related to the individual distribution uniformity, therefore it caused the phenomenon that Shannon-Wiener diversity index reduced by adding activated carbon.

Figure 2. Shannon-Wiener diversity index of soil seed bank by adding different inorganic substrates.

3.3. Dynamic curve of seed germination
Several groups with high seed germination density were selected as the research objects, in order to explore the effect of different ratio of inorganic substrates (activated carbon, perlite and vermiculite)
on seed germination. As shown in figure 3, it could be seen that the germination curve generally presented “S” shape, and with “smooth growing-rapid growing-stable” period, but the time for each period differed depended on different inorganic substrates. Compared to the others treatment, the topsoil mixed with 40% vermiculite had very significant effect on the late period of seed germination. In addition, the number of seed germination in blank control group was more efficient on germination density in the period of 3/16 till 3/26. On the one hand, it was because the blank control group didn't contain any inorganic substrates, and the content of topsoil was higher than other groups, thus the seeds in the topsoil was relatively higher; while the content of topsoil in other groups relatively less, so the number of seeds was reduced; In other hand, with the time of seed germination and seedling growth, the soil nutrients in germination disk decreased gradually, while the inorganic substrates played a multiple action on water conservation and mineral fertilizer. Therefore, the effect of inorganic substrates on the germination of soil seed bank was mainly reflected in the late stage of germination.

![Germination curve of soil seed bank by adding different inorganic substrates.](image)

**Figure 3.** Germination curve of soil seed bank by adding different inorganic substrates.

4. **Conclusion**

Based on the analysis of the above, inorganic substrates promoted the soil seed bank density and species diversity in a certain extent. When vermiculite mixed with topsoil in 40%, its germination effect of soil seed bank was obvious, the density of soil seed bank reached $6 \times 10^5$ plants/m$^2$, Shannon Wiener diversity index reached 1.4354. Therefore, it was more conducive to improve the soil seed bank density and species diversity by adding 40% vermiculite in topsoil. But the promoting effect on seed germination by adding inorganic substrates was not highly significant. This may related to the specific nature of the inorganic substrates, although the chemical properties of inorganic substrates was relatively stable, but its fertilizer retention capacity and buffering capacity was relatively poor, so its effect on the promotion of seed germination was not particularly significant. Therefore, adding substrates can be used as one of artificial measures for soil seed bank application in vegetation restoration project, which needed deeper study in germination characteristic of soil seed bank by adding more other type substrates, so as to offer reference to engineering application in soil seed bank.

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