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Role of nurse shrubs for restoration planting of two conifers in southeast of Mu Us Sandland, China

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Abstract

Two-year-old pine seedlings, *Pinus tabulaeformis* and *Pinus sylvestris* were planted under the canopies of three shrub species and in open areas to test for facilitation during seedling establishment in southeast of Mu Us Sandland in northern part of Shaanxi, China. Pine seedlings establishment were assessed three times within three consecutive growing seasons. Height, area and volume of shrubs were measured. Microclimate conditions (light intensity, air temperature and soil temperature and moisture) were recorded in four microhabitats. Near surface light intensity, air temperature and soil temperature were lower under shrubs, which led to higher soil moisture and pine seedlings under the canopy of shrub species. Pine seedlings survival was remarkably higher when planted under the canopy of shrub species (65.7% for *P. tabulaeformis* and 60.6% for *P. sylvestris*) as compared with open areas (22.4% for *P. tabulaeformis* and 38% for *P. sylvestris*). *P. tabulaeformis* with shade-tolerance trait expressed high survival of seedlings as compared to that of *P. sylvestris* seedlings under the canopy of shrub species (Tukey test, $P < 0.05$). Leguminous shrub (*Caragana korshinskii* and *Amorpha fruticosa*) showed continuously facilitation during moderate drought stress (summer 2012, 2013 and 2014), but dense and small shrub (*Caragana korshinskii*) reduced the establishment of seedlings possibly for light competition. *Salix cheilophila* showed a facilitation effect in growing seasons, but the effect of allelopathy led to high mortality of seedlings under their canopy. In addition, two pine growths were not inhibited when planted under three shrubs. In conclusions, nurse-shrub facilitation can be used as an effective restoration strategy in this sandland. However, use of shrubs as nurse plants depends on their canopy structure and ecological impacts; the selection of target species depends on their shade tolerance traits.

Key words

Facilitation, Mu Us Sandland, Nurse shrub, Pine, Restoration

Introduction

Mu Us Sandland is a classical semi-dry zone in China. The drys ecosystems have much sensitive response to global change there (Xiao, 2001). Due to serious desertification caused by over-grazing, mining and inappropriate agricultural-management practices combined with repeated drought, it has been degraded to desert conditions, so that the possibility of their natural recovery has declined significantly (Wu, 2002; Ohte *et al.,* 2003). Hence, there is a vital need for restoration of these sandland to attain more sustainable ecosystems. The most crucial stage is seedling establishment in restoration project. In Mu Us Sandland, it can largely be limited by soil moisture deficiency, temperature fluctuation, high light intensities, low soil fertility , competition, allelopathy, herbivory and wind (Jankju, 2013). Several restoration techniques are used to alleviate this problem. Nevertheless, use of any of these techniques is restricted because of low survival and high cost (Maestre *et al.,* 2001; Andel *et al.,* 2005). Thus, there is a need for developing novel, low-cost and efficient restoration techniques.

Interaction effects by nurse plants can significantly increase or decrease seed germination and seedling establishment under their canopies (Soliveres *et al.,* 2010; Jankju， 2013). Several experiments in the Mediterranean regions have shown successful use of nurse shrubs in restoration projects (Rousset and Lepart, 2000; Castro *et al.,* 2002 and 2004; Gómez-Aparicio *et al.,* 2004; Sanchez-Velaquezet *et al.,* 2004). The "stress-gradient" hypothesis (Callaway, 2007) reveals that shrub facilitation for understory plants should be more common in desert ecosystems than the Mediterranean climates. Nevertheless, there are debates on the role of shrubs in arid and desert ecosystems. Several authors have reported higher plant diversity, emergence and/or survival rate under the canopy of shrubs, as compared with open areas (King, 2008; Padilla and Pugnaire, 2006), while others have reported contrasting results (Anthelme and Michalet, 2009; Huber-Sannwald and Pyke, 2005; Tielbörger and Kadmon, 2000). Therefore, the primary aim of the present study experiment was to test the capability of shrubs as a restoration tool in the southeast of Mu Us Sandland.

Selection of the best nurse species is an important decision in restoration projects, as this will determine the success of the project (Gómez-Aparicio *et al.,* 2004; Sanchez-Velaquezet *et al.,* 2004). Previous studies on the role of nurse plants as a restoration tool have shown that the nursing effects can be dependent on varying levels of shading created by nurse plants (Hastwell and Facelli, 2003;Sanchez-Velaquez *et al.,* 2004)and morphological characteristics of the nurse shrubs (Callaway, 2007). Furthermore, nitrogen-fixing capability may enhance positive interactions (Callaway, 2007), whereas allelopathic shrubs (Gómez-Aparicio *et al.,* 2004) may enhance negative plant-plant interactions. Accordingly, the second aim of the present study was to compare nursing effects of three sandland shrub species, which were different in terms of canopy architecture (loose vs. dense), canopy size (large vs. small) and ecological impact (N-fixing and allelopathy) on seedling survival of two pine species.

Interaction among plants depended upon species characteristics, so selection of target species (ie those being restored) may influence the outcome of restoration project (Padilla and Pugnaire, 2006). Furthermore, the balance of an interaction could be determined by the ecological requirements of the species. (Liancourt *et al.,* 2005). Gómez-Aparicio *et al.* reported that shade-tolerant species showed a more positive effect in response to nurse plants than shade- intolerant trees (Gómez-Aparicio *et al.,* 2004). Accordingly, the third aim of this research was to compare positive effect of two pines (*P. tabulaeformis* and *P. sylvestris)* in response to nurse shrubs, which were different in terms of shade-tolerant traits.

Materials and Methods

Study area : The study was conducted at The Sandland Ecological Station of Yulin University, located in the southeastern of Mu Us sandland in northern part of Shaanxi province, China. The climate was temperate continental dry, with mean annual temperature of 7.8~8.6 ºC and average annual precipitation of 260-450 mm, which is concentrated in the summer (7~9 months), following a prolonged spring drought. Soil texture was sandy clay loam. In area, sand movements are frequent, the type of vegetation pushed toward desert vegetation dominated by shrubs and herbs (Yu *et al.,* 2004).

Plant species : The potential nurse effect of three dominant shrub species were analyzed in southeast of Mu Us sandland: a possible allelopathic effects shrub with a wide and loose canopy (*Salix cheilophila* Schneid, hereafter *Salix*), and two different-sized leguminous shrubs (*Caragana korshinskii* kom, hereafter *Caragana*) and *Amorpha fruticosa* L, hereafter *Amorpha)*. *Pinus tabulaeformis* and *Pinus sylvestris* with different shade tolerant traits were widely used for restoration of southeast of Mu Us sandland. The two pine seedlings were target species to be planted under the canopy of shrubs or in open areas. Pine seedlings used in the experiment came from a nursery in the Forest Seed and Seedling Station of Yulin City, and was 2 year old. Pine seedlings were grown in beds before transplanting. At the time of reforestation, they were bare-root transplanted.

Experimental design : To test the role of nurse shrub for restoration planting of two pines, four treatments were selected. Each shrub species was considered as a separate treatment (three shrubs, three microhabitats), and "open areas" between shrubs was a control treatment (the fourth microhabitats). The experimental pine seedlings, planted between 14^{th} to 24^{th} March, 2012 were distributed in three 3,000-m^2 plots separated by approximately 200 m. In each plot, 30 seedlings per pine species per microhabitats (720 experimental seedlings per pine species in total: 30 sampling points × 2 pine levels × 4 microhabitats × 3 plots) were planted, sampling points being randomly assigned. In June 2012, before the beginning of summer drought, the planted pines that had died were excluded from the experiment.

Microclimate measurements : Several microclimate conditions influencing seedling establishment and survival were measured simultaneously on 5 replicates of each shrub species and open areas. Light and temperature at the top 10 cm of the soil layer was measured using a light intensity and temperature auto-recorder (Hobo Pendant). At the same time, soil temperature data were collected at 15min intervals from 10a.m. to 4p.m with Hobo Pendant. Finally, soil moisture was measured 10 cm below the soil surface, using a Hydra Soil Moisture Probe. All microclimate variables were measured in the same randomly chosen plot.

Pine and shrubs morphology : For each experimental pine, the survival was sampled and recorded three times: after first (August 2012), second (August 2013) and third summer (August 2014). The length of the leader shoot was also recorded in the summer. Shrubs' morphology was studied on 20 random samples of each species. A preliminary field survey indicated canopy surface of all three shrub species to be very close to a rounded shape. Therefore, the longest diameter of each shrub was measured, for estimating maximum canopy area. Further, height of tallest branch of each shrub was measured, for estimating maximum height and volume.

Data analysis : Generalized Linear Modeling (GLM), with repeated measures, was applied in the data for pine survival and length of the leader shoot, using SPSS 13 statistical program. For this, measurement date (summer 2012, summer 2013, and summer 2014) was considered as random factor and microhabitat (understory of three shrub species and open areas) was considered as the main factor. Mean values for soil moisture, soil temperature, near surface temperature and light intensity, three canopy height and area and seedling establishment in four microhabitats were compared by Tukey's test.

Results and Discussion

Significant differences were observed between numbers of established pine seedlings under the shrub canopies and those in open areas (Fig.1). The overall pine survival rate after three growing seasons was 65.7% for *Pinus tabulaeformis* and 60.6% for *Pinus sylvestris* under the canopy of shrubs, values that were remarkably higher than those found in open area (22.4% for *Pinus tabulaeformis* and 38% for *Pinus sylvestris*). This result indicated that survival of planted pines can be promoted under all shrubs in comparison with open areas.

The nurse species that impart greater micro-environmental benefits, such as increasing shade, reducing summer-time radiation load, increasing night-time winter temperature, increasing soil moisture and reducing herbivory, had higher facilitation impact on their understory plants (Callaway, 2007; Jankju， 2013). In this experiment, all shrubs significantly reduced irradiation under their canopy as compared to open areas. Light intensity was higher under *Amorpha* canopy than under the canopy of any other shrub species. Open area had highest values (Fig.4a). Air temperature near soil surface was generally lower under the canopy of shrubs than in open areas.

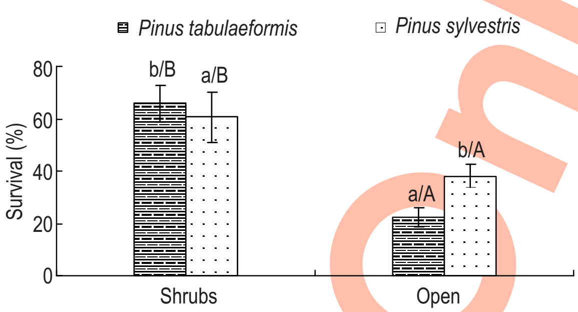

Fig 1 : Total survival rate of *Pinus tabulaeformis* and *Pinus sylvestris* seedlings under the canopy of shrub species or in open areas. Mean values ±SE are shown, different alphabetic letters indicate significant differences by Tukey test (P＜ 0.05, df=1). Lowercase indicates difference among different treatments in same group, and capital indicates difference of same treatment between two groups.

The warmest understory was for *Amorpha* (Fig.4b). Instantaneously measured soil temperature followed similar trend (Fig. 4c). Shrub also differed in their effects on soil moisture. Significantly higher soil moisture was found under canopy of *Salix*. *Caragana* and *Amorpha* retained slightly higher (non-significant difference) soil moisture under their canopies as compared to nearby open areas (Fig. 4d). These results show that near surface light intensity, air temperature and soil temperature were lower under shrubs, which led to higher soil moisture and pine seedlings under the canopy of shrub species.

Growth of leader shoot differed between measurement date for two pine species (Table 2), being lowest during the first growing seasons (4.8±0.3vs. 4.5±0.5cm for *Pinus tabulaeformis*; 7.2±0.3 vs. 4.2±0.2cm for *Pinus sylvestris*). The growth of *Pinus tabulaeformis* was similar in all microhabitats (Table 2; 7.8±0.2cm on average after three growing season). For *Pinus sylvestris*, however, growth was affected both by measurement date and microhabitat (Table 2), with significant interactions between the two factors, indicating that the effect of microhabitat was dependent on date. The growth of *Pinus sylvestris* was highest in *Amorpha* and open area (9.8±0.1cm; 10.9±0.3cm), lowest in *Caragana* (5.6±0.2cm). This result showed that shoot growth of planted pines could not be reduced under all shrubs in comparison with open areas.

Results of GLM analysis (Table 1) revealed significant effects of microhabitat (shrubs) on the survival rates of *Pinus*

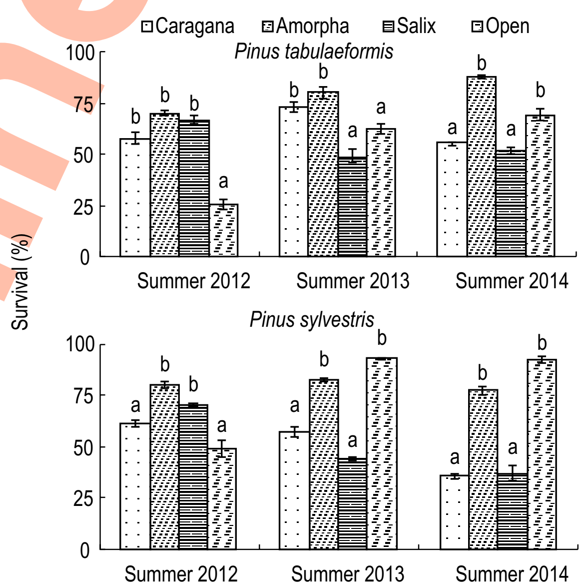

Fig. 2 : Survival rate of *Pinus tabulaeformis* and *Pinus sylvestris* seedlings under the canopy of shrub species or in open areas in three consecutive growth seasons. Mean values ±SE are shown, different alphabetic letters indicate significant differences by Tukey test (P＜0.05, df=3)

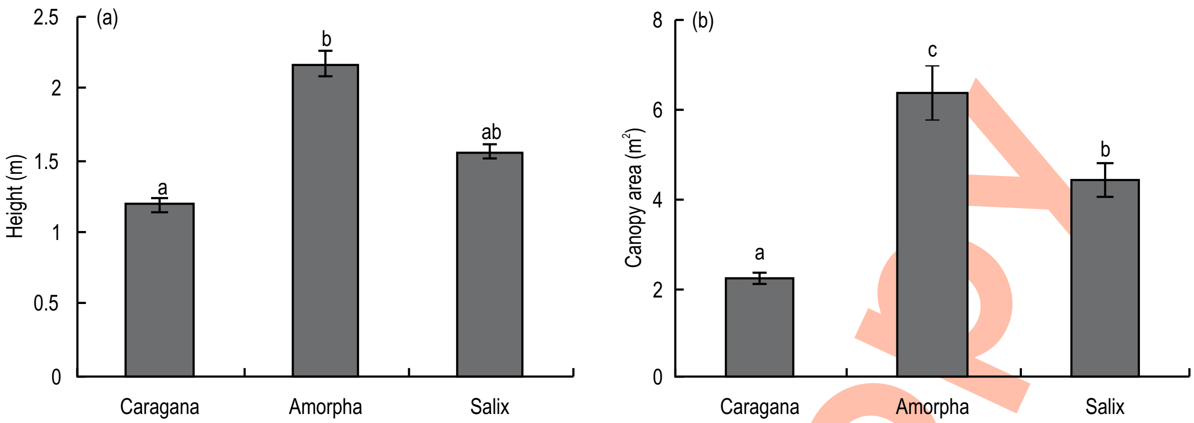

Fig. 3 : Canopy height of shrub species. Mean values ±SE are shown, different alphabetic letters indicate significant differences by Tukey test ($P < 0.05$, df=2)

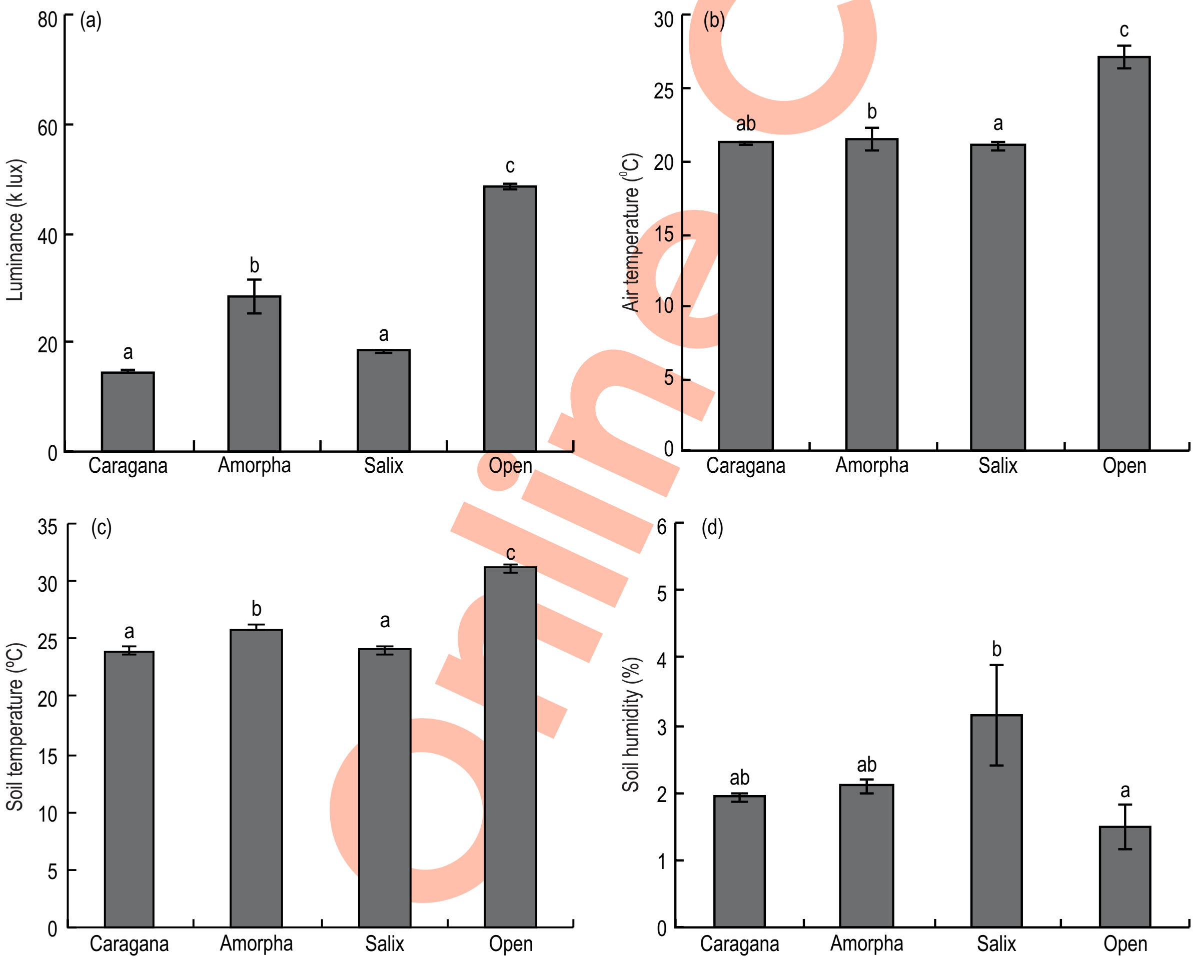

Fig. 4 : Microclimate conditions under the canopy of shrub species or open areas, light intensity (a), air temperature (b), soil temperature (c) and soil moisture (d) are compared. Mean values ±SE are shown, different alphabetic letters indicate significant differences by Tukey test ($P < 0.05$, df=3)

tabulaeformis and *Pinus sylvestris* seedlings. However, interaction between factors was not significant, which indicated shrub effects were similar at different dates during the experiment (*i.e.* spring 2012 till summer 2014).

At the end of first growing season (summer 2012), there were significant observed differences between numbers of established seedlings under the shrub canopies and those in open areas (Fig. 2). At this date, two pine seedlings establishment was highest under *Amorpha* and *Salix* species, whereas the number of established *Pinus sylvestris* seedlings under the canopies of *Caragana* was similar to that of open areas. At the end of second and third growing season (summer 2013 and 2014), two pine seedlings establishment under *Amorpha* species was similar to that of open areas. However, some pine seedlings were dead under the canopies of *Caragana* and *Salix*. Furthermore, survival of established two pine seedlings under the canopies of *Caragana* and *Salix* were significantly lower than in open areas.

The difference between the effects of shrub was possibly due to the differences in their canopy structure and ecological impacts (i.e. N-fixing versus allelopathy). A general trend among plant-plant interaction for small and allelopathic was a directional reduction in facilitation during the time course of the experiment. However for N-fixing shrub, it was positive during experiment (Maestre and Cortina, 2004). In the present study, *Salix* was allelopathic shrub, while *Caragana and Amorpha* was N-fixing shrub. The widest canopy crown was found in *Amorpha* and smallest in *Caragana*, with those of *Salix* being intermediate (Fig. 3a). Difference in shrub heights were similar to those for canopy area (Fig.3b).

A loose canopy structure for *Amorpha* led to a brighter and warmer microclimate under its canopy as compared with other shrubs (Fig. 4a-b-c). Furthermore, a taller and greater canopy area (Fig. 3) can provide a more stable microenvironment under the canopy, as compared to other shrub species (Gomez-Aparicio, 2009; Maestre and Cortina, 2004). Therefore, more favorable and stable microclimate conditions were possible reasons for higher two pine seedlings establishment, under the canopy of *Amorpha* than in open areas, as compared with the canopy of *Caragana* and *Salix* species (Fig. 2). A dense canopy structure for *Caragana* led to a lower light intensity, near surface air temperature and soil temperature under its canopy as compared with other shrubs (Fig. 4a-b-c). Canopy area and height of *Caragana* was small as compared with that of other shrubs (Fig. 3). Two pine seedling establishments were significantly lower under *Caragana* than other treatments. Therefore, the major limiting factor for early establishment of two pine seedlings under the canopy of *Caragana* was possibly the lower light intensity. Several researchers have reported the negative effects of varying levels of shading created by different nurse plants on seedling establishment of target species (e.g. Gómez-Aparicio *et al.*, 2004; Sanchez-Velaquez *et al.*, 2004).

Plant-plant interaction becomes more complicated when the nurse plants have allelopathic effects (Gómez-Aparicio *et al.*, 2004). Early in growing season (summer 2012), *Salix* facilitated establishment of pine seedlings under its canopy. Such positive effects could be due to a more stable microclimate conditions, under its canopy as compared with open areas. Although lack of specific data, one might expect that the allelopathic effects of *Salix* would be low, early in the growing season. Later in the

Table 1 : Results of GLM analysis with repeated measurements on survival of *Pinus tabulaeformis* and *Pinus sylvestris* seedlings

Test types	Sources of variation	*Pinus tabulaeformis*			*Pinus sylvestris*	
		df	F	Sig.	F	Sig.
Within-subjects	Date	2	152.783	.072	202.548	.053
	Date ×Microhabitat	7	2.656	.081	4.367	.066
	Error	191				
Between-subjects	Intercept	1	464.814	.000	513.642	.000
	Microhabitat	3	4.432	.013	6.142	.002
	Error	96				

Table 2 : Results of GLM analysis with repeated measurements on leader shoot length of *Pinus tabulaeformis* and *Pinus sylvestris* seedlings after three growing seasons

Test types	Sources of variation	*Pinus tabulaeformis*			*Pinus sylvestris*	
		df	F	Sig.	F	Sig.
Within-subjects	Date	2	38.82	.002	20. 48	.003
	Date ×Microhabitat	7	5.65	.078	4.367	.036
	Error	204				
Between-subjects	Intercept	1	4.81	.042	9.07	.000
	Microhabitat	3	1.83	.054	6.14	.014
	Error	350				

growing season, the allelopathic effect of *Salix* was probably intensified. As a result, seedling mortality rate was much higher under the canopy of allelopathic shrubs (*Salix*) as compared with non-allelopathic (*Caragana* and *Amorpha*).

There were significant differences observed between numbers of established *Pinus tabulaeformis* and *Pinus sylvestris* seedlings under the shrub as canopies or in open areas (Fig.1). *P. tabulaeformis* expressed high survival of seedlings compared to that of P. sylvestris seedlings under the canopy of shrub species (Tukey test, $P < 0.05$), whereas the number of established *P. sylvestris* seedlings was high as compared to that of *P. tabulaeformis* in open areas (Tukey test, $P < 0.05$). In spite of this positive influence, the nurse effect may be insufficient to increase plant establishment if target species have a low tolerance for the prevalent abiotic conditions (Padilla and Pugnaire, 2006). In the present study *Pinus tabulaeformis* with shade-tolerance trait expressed high survival of seedlings as compared to that of *Pinus sylvestris* seedlings.

In conclusion, changes in light/air temperature/soil temperature/soil moisture under the shrub story had positive impact on two pine seedlings in the present study. The positive effects of shrubs on the survival of pine suggest that nurse shrub can be used as an effective restoration strategy in the southeast of Mu Us Sandland. However, use of shrubs as nurse plants depends on their canopy structure and ecological impacts; selection of plants as target species depends on their shade tolerance traits.

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