Afforestation of the Gobi Desert: A Nursery Protocol for Producing High-Quality *Haloxylon ammodendron* Seedlings

Enkhchimeg Tsedensodnom, Ser-Oddamba Byambadorj, Khaulenbek Akhmadi, Gabriella S. Scippa, Donato Chiatante, Antonio Montagnoli, Batkhuu Nyam-Osor, and John A. Stanturf

PhD student, Laboratory of Forest Genetics and Ecophysiology, School of Engineering and Applied Sciences, National University of Mongolia and Institute of Geography and Geoecology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia; PhD student, Laboratory of Forest Genetics and Ecophysiology, School of Engineering and Applied Sciences, National University of Mongolia, Ulaanbaatar, Mongolia, and Department of Environment and Forest Resources, College of Agriculture and Life Science, Chungnam National University, Daejeon, Republic of Korea; Researcher, Institute of Geography and Geoecology, Mongolian Academy of Sciences, Ulaanbaatar, Mongolia; Professor, Department of Biosciences and Territory, University of Molise, Isernia, Italy; Professor, Department of Biotechnology and Life Science, University of Insubria, Varese, Italy; Researcher, Department of Biotechnology and Life Science, University of Insubria, Varese, Italy; Professor, Laboratory of Forest Genetics and Ecophysiology, School of Engineering and Applied Sciences, National University of Mongolia, Ulaanbaatar, Mongolia; Visiting Professor, Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Tartu, Estonia and Senior Researcher, InNovaSilva, Vejle, Denmark.

Abstract

Conservation and restoration of degraded arid lands in Mongolia depend upon protecting the remnant forests from further degradation and restoring denuded sites. Worsening environmental conditions, driven by climate change, intensify the need for active restoration. *Haloxylon ammodendron* (C.A. Mey.) Bunge. is a candidate plant species for restoration, but low water availability constrains its germination and establishment. Thus, active restoration requires massive production of high-quality seedlings. Here we present a nursery protocol for producing high-quality container seedlings with high percentages of germination and seedling establishment. We tested three representative field soils as growing media with three watering regimes. All treatment combinations produced high-quality seedlings, though there were differences in shoot height, root collar diameter, shoot and root biomass, and root length. We conclude that field soils reflective of outplanting conditions can be used for seedling production, and the nursery watering regime can be adapted to local conditions if a minimum moisture level can be maintained.

Introduction

Arid lands characterize more than 40 percent of the Earth's land surface, contribute 40 percent to global net primary productivity, and support more than 38 percent of humanity (Wu et al. 2019). Climate change threatens these sensitive ecosystems (Li et al. 2019, Huang et al. 2016) by causing severe and widespread droughts (Dai 2013) that often lead to desertification (D'Odorico et al. 2013). It is not surprising, therefore, that desertification affects drylands of central Asia (Byambadorj et al. 2020, Mandakh and Dash 2013, Nyam-Osor et al. 2021). These biodiversity hotspots (Leadley et al. 2010) are highly sensitive to climate change (Seddon et al. 2016).

In the absence of reduced greenhouse gas emissions (Lioubimtseva and Henebry 2009), sustainability of central Asian ecosystems is at risk. Revegetation efforts (Wu et al. 2019) could halt (or reverse) desertification (Zhang et al. 2016) and mitigate climate change by sequestering carbon in plant biomass (Schulze et al. 2000). Restoration initiatives in central Asia use the genus *Haloxylon*, known as the "forest of the desert" (Jia et al. 2008, Li et al. 2019, Zhu and Jia 2012), given its broad distribution in middle and central Asia, from northwest China to Afghanistan and Iran (Buras et al. 2012, Guo et al. 2005, Thevs et al. 2013). Species of *Haloxylon* occur as tall shrubs or small trees (figure 1). These plants prefer sandy soils (Khassanov et al. 1994); growth and survival decrease with increasing presence of silt and clay in the soil (Kayo 2019). *Haloxy-*

Figure 1. *Haloxylon ammodendron* occurs as tall shrubs or small trees in the Gobi Desert region of Mongolia. (Photos by Ser-Oddamba Byambadorj)

lon trees present xeromorphic and halophytic adaptations (Chang et al. 2019) by regulating water uptake and transpiration (Gong et al. 2006, 2015; Höhl et al. 2020; Ma et al. 2007; Xu et al. 2007). *Haloxylon* trees have deep roots (Wei et al. 2007) and branches with high water-use efficiency that limit photorespiration and transpiration (Ashraf and Harris 2013). These characteristics lead to increased photosynthetic yield (Doubnerová and Ryšlavá 2011, Sage 2004, Sage et al. 2012).

Despite adaptations to dry climates, *Haloxylon* species are at risk (Enkhchimeg et al. 2020, Lioubimtseva and Henebry 2009) due to extensive harvesting for pharmaceuticals and firewood (Jiang and Tu 2009) and to the use of succulent stems as fodder for livestock (Akhani et al. 1997, Song et al. 2006). Since 1980, *Haloxylon* species abundance has decreased by 70 to 80 percent in Turkmenistan, Kazakhstan, and Uzbekistan (Rachkovskaya 2003, Rachkovskaya et al. 2003, Rathore et al. 2012, Thevs et al. 2013).

Mongolia supports 2 million ha of *Haloxylon* forest in the arid southern region (Dorjsuren 2009), representing 13 percent of the total national forest inventory. Even though half of this forest is protected, significant degradation has occurred (Khaulenbek et al. 2018). Thus, the Government of Mongolia supports several forest restoration initiatives, including the Mongolia-Korea Joint Green Belt Plantation Project (Stanturf et al. 2020) that has already restored 1,490 ha of desert land during the last decade (Khaider et al. 2019).

Physiological and morphological studies show that *Haloxylon* trees absorb groundwater at great depth (Yang et al. 2007) when they have reached maturity (Sheng et al. 2004). During the initial phase of seedling growth, surficial water from rainfall evaporates rapidly, contributing to poor seed germination and poor survival of *Haloxylon* (Meshkov et al. 2009). Therefore, successful restoration in these habitats may be improved through use of nursery-grown seedlings, which tend to have higher establishment rates compared with seed (Middleton 1998, Nosrati et al. 2013). Although production of high-quality seedlings requires more resources initially, improved field performance can offset these costs (Haase et al. 2021).

Several field studies have investigated germination, emergence, and establishment of *Haloxylon* seedlings (Li et al. 2017; Song et al. 2005, 2006; Tobe et al. 2004, 2005), but few (Wang et al. 2019) have focused on nursery practices to produce high-quality *Haloxylon* seedlings. The objective of this study was to evaluate germination and development of nursery-grown *Haloxylon* seedlings grown in three field soils collected from different desert sites using three watering regimes.

Materials and Methods

Experimental Site

We conducted this study at the forest nursery of the Korea-Mongolia Joint Green Belt Plantation Project located at Bayanzag (44°5'25.73"N, 103°42'38.47"E) at an altitude of 1100 m in Bulgan soum (Dal bag), Umnugobi province, 675 km south of the capital Ulaanbaatar. Bayanzag belongs to the East Gobi semi-desert steppe region (Grubov 1982). Mean annual precipitation in the area is 137 mm (measured at Saikhan station in Bulgan soum), with 80 to 90 percent occurring in the summer and autumn months. Mean annual air temperature is 6.2 ± 1.1 °C, and summer average temperature is 19.8 ± 0.45 °C. The mean air temperature of the warmest month (July) is 24.2 °C, while that of the coldest month (January) is -13.8 °C (NAMEM 2019). The growing season starts in May and ceases at the end of September with the first autumnal frosts. Annual temperature and precipitation were measured during the 3-year study using a HOBO H21-USB Micro Station (Onset Computer Corp, USA) (figure 2).

Seed Collection and Germination Assessment

Bulk seed samples were collected in December 2013 from natural *Haloxylon ammodendron* (C.A.Mey.) Bunge. stands in the Gurvantes region of the Umnugobi province (43°27'55"N, 101°16'35.77"E). Seeds were stored in airtight containers and refrigerated at 5 °C for 5 months until the experiment began in spring 2014. Seed-quality traits were determined according to the Mongolian National Standard (MNS 2887: 2009) and included germination capacity, germination energy, and 1,000-seed weight (g). Germination capacity and energy were determined on 4 replicates of 50 seeds, each sown on filter paper in 9-mm plastic petri dishes. Deionized water was added to each dish until half the volume of each seed was immersed (about 5 ml). The Petri dishes were covered with lids and held for 8 to 16

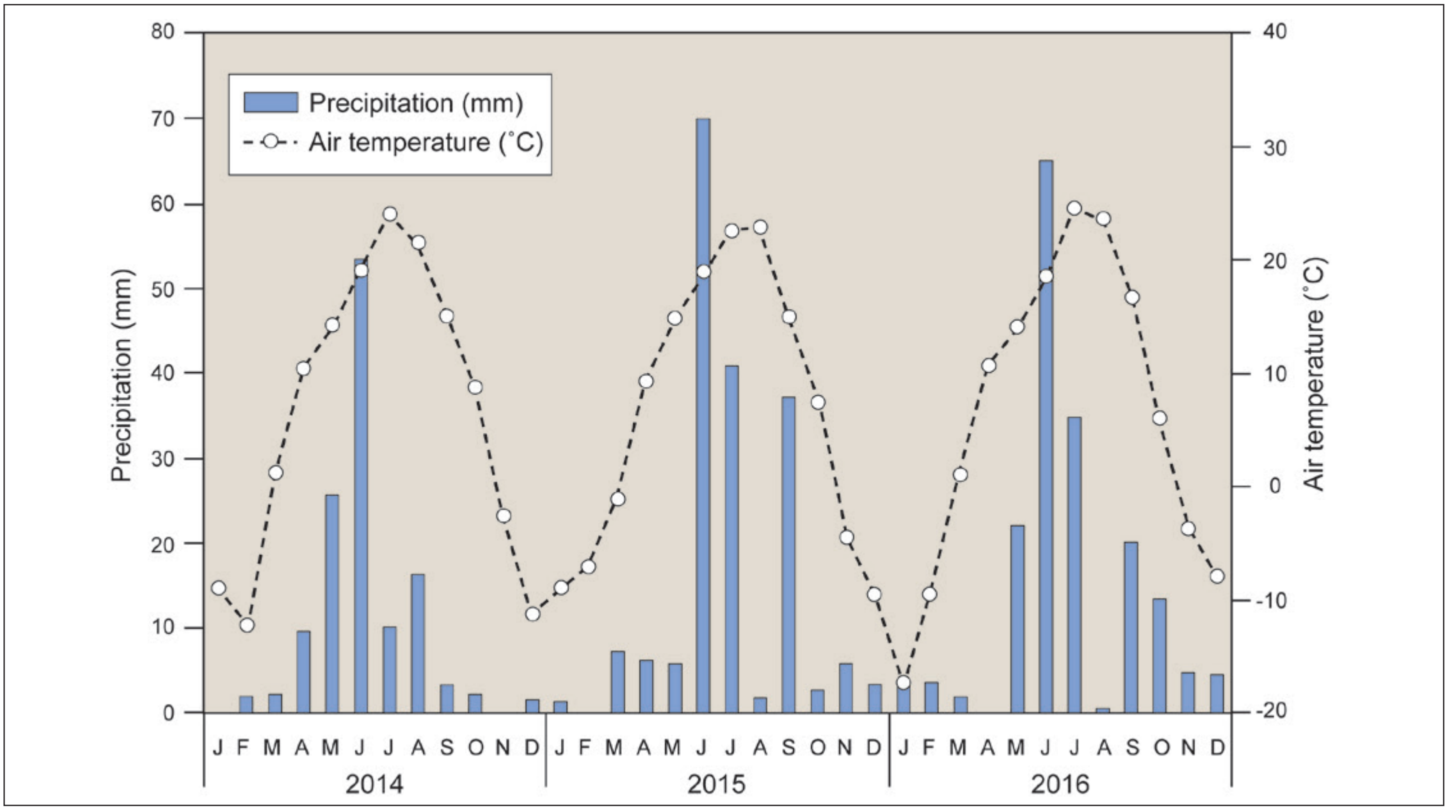

Figure 2. Average monthly temperature and precipitation during the experiment were measured on site.

h at 25 °C under continuous illumination (3000 lux) in a plant growth chamber (GC-300TL, Lab Companion, JEIO TECH Co. Ltd.). Seeds were considered germinated when the radicles emerged. Germinated seeds were counted and removed daily. Germination capacity is the proportion of total germinated seeds to total sown seeds and is expressed as a percentage. Germination energy (also expressed as a percentage) is one of the commonly employed indices of germination speed (ISTA 1999) and was computed as the proportion of total germinated seeds after 3 days to that of total germinated seeds after 5 days.

Soil Type and Analysis

Three soil types were used to evaluate emergence and seedling development. The soil types used are commonly distributed in the study region (table 1). The most dominant soil types are Arenic Yermic Calcisols (in desert areas) and Arenic Calcisols (in saxaul forested areas) (Batkhishig 2016, FAO 2015). Soils were collected from their respective regions to a depth of 20 cm and used without any further processing. Each soil type was used to fill 300 polybag containers (10 by 20 cm; 37.7 cm^3 volume) for a total of 900 containers in the study.

Each of the three soil types were sampled, air dried, sieved to 2 mm, and analyzed for physical and chemical properties (ISO 2006). Particle size composition was determined by the pipette method (Burt 2004), soil organic matter (SOM) by the oxidation method of Walkley and Black (Nelson and Sommers 1996), calcium carbonate content by the volumetric method (ASTM 1996), pH by using a glass electrode pH meter on a 1:2.5 air-dried soil/distilled water mixture (MNS ISO 10390: 2001), and electrical conductivity (EC) by using a platinum electrode on a 1:5 air-dried soil/distilled water mixture. Available phosphorus (P_2O_5) was measured by molybdenum blue colorimetry after ammonium carbonate digestion (MNS 3310: 1991). Nitrate-nitrogen (NO_2-N) was determined by sodium acetate digestion and spectrocolorimetry. Potassium (K_2O) was analyzed by flame spectrometry (Burt 2004).

Emergence Assessment

In April 2014, 6 seeds were sown in each of 900 polybag containers at a depth of 0.5 cm. The total percentage of seedlings emerged 4 weeks after sowing was recorded. Emergence rate was estimated by a

Table 1. Physical and chemical analysis of three local soil types used as growing media for *Haloxylon ammodendron* seedlings.

		Soil type		
Characteristic	**Units**	**Gobi red soil-Ferric Calcisols (Soil 1)**	**Gobi sandy loamy light-brown soil-Arenic Yermic Calcisols (Soil 2)**	**Sandy Gobi brown soil-Arenic Calcisols (Soil 3)**
		Parameters		
pH	water	8.643	8.133	8.597
$EC_{2.5}$	dS/m^{-1}	1.385	0.139	0.250
$CaCO_3$	%	0.270	8.117	0.420
Organic matter	%	0.113	1.555	0.261
$N\text{-}NO_3$	mg//kg	0.707	0.467	1.740
		Soil texture		
Sand	%	74.73	60.83	82.97
Silt	%	17.56	24.73	9.61
Clay	%	7.72	14.45	7.42
		Soluble anions		
CO_3^{2-}	mg/100gr	0.000	0.000	0.000
HCO_3^-	mg/100gr	1.930	1.223	1.173
Cl^-	mg/100gr	1.650	0.247	0.467
SO_4^{2-}	mg/100gr	8.693	0.160	0.517
		Soluble cations		
Ca^{++}	mg/100gr	2.073	1.168	1.173
Mg^{++}	mg/100gr	0.537	0.287	0.173
Na^+	mg/100gr	9.607	0.148	0.777
K^+	mg/100gr	0.055	0.028	0.035
		Mobile		
P_2O_5	mg/100g	3.020	2.057	4.663
K_2O	mg/100g	4.873	6.960	8.363

modified index of germination rate (Rozema 1975) as follows:

$$\text{Emergence rate} = \sum \frac{100Gi}{nti}$$

where n is the total number of seeds and Gi is the number of seedlings emerged on day ti (ti=0, 1, 2, 3 ....).

During emergence assessment, containers were irrigated every 3 days for 25 min with a soaker hose (Smile Spray Hose, SML 6-6 Namkyung Company Ltd) capable of delivering 18 L min^{-1}. At the end of the 4-week emergence assessment, a single seedling (healthy and undamaged) was left in each container to be used for evaluation of seedling growth in response to soil and watering treatments.

Watering Regimes

After seedling emergence (May 2014), containers were watered to field capacity every 3 days for 1 month to assure seedling establishment. After establishment, three watering regimes (low, medium, and high) were applied to each of the three soil types by irrigating for 25 min at intervals of 3, 7, or 14 days (Nyam-Osor et al. 2014; 2018). Irrigation was applied during the growing season (May to September) for 3 years (2014 through 2016) (figure 3). For logistical reasons related to the automatic watering system, seedlings assigned to each treatment combination were kept together in a group, though environmental conditions throughout the study area were otherwise fairly uniform.

Seedling Measurements

At 10-day intervals throughout the 3 growing seasons, 50 seedlings were randomly selected from each treatment group and measured for height and root collar diameter (RCD). At the end of the experiment (September 2016), 15 randomly selected, healthy seedlings per treatment were harvested and measured for shoot height, RCD, root length, fresh weight, and dry biomass. Shoot height was measured from the root collar to the tip of the young shoot. Root length was measured from the root collar to the tip of the tap root. RCD was measured with a digital Vernier caliper at the base of the stem (Thompson and Schultz 1995). All harvested seedlings were separated into shoots and roots. Soil particles were rinsed from the roots. After fresh weight was measured, root and shoot samples were oven dried at 80 °C for 48 h and weighed for dry biomass (Cregg and Zhang 2001).

Statistical Analysis

The experimental design for this project was a completely randomized design with 100 replications (polybag containers) of 9 treatments consisting of a 3 by 3 factorial (soil type by watering regime) (figure 4). Irrigation logistics constrained arrangement of the containers, but we are confident that environmental gradients did not have an influence in the relatively uniform study area. The SAS version 9.4 software package (SAS Institute Inc. 2014) was used to analyze the data with two-way analysis of variance

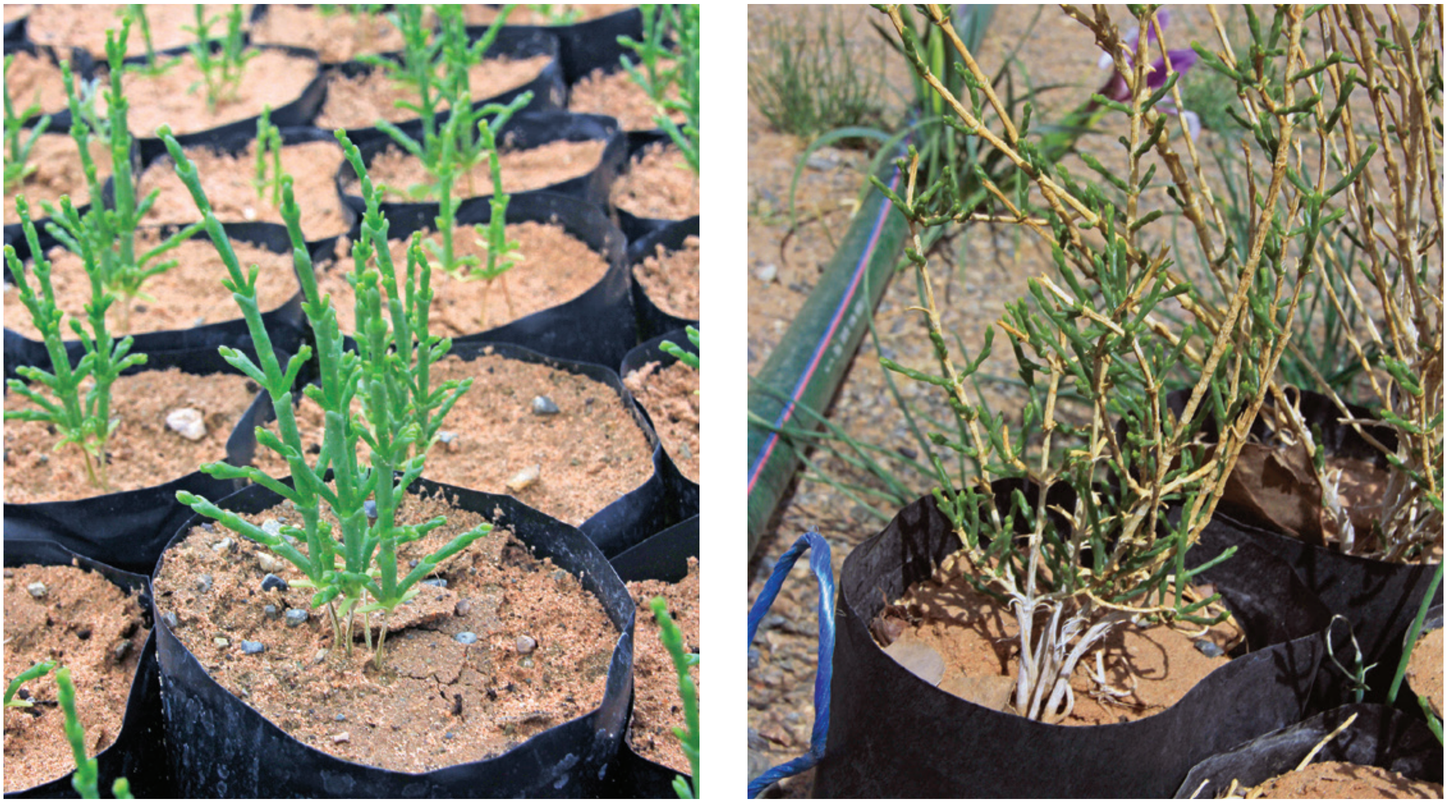

Figure 3. *Haloxylon ammodendron* seedlings were assessed for 3 years to evaluate development in response to soil type and watering regime. (Photos by Ser-Oddamba Byambadorj)

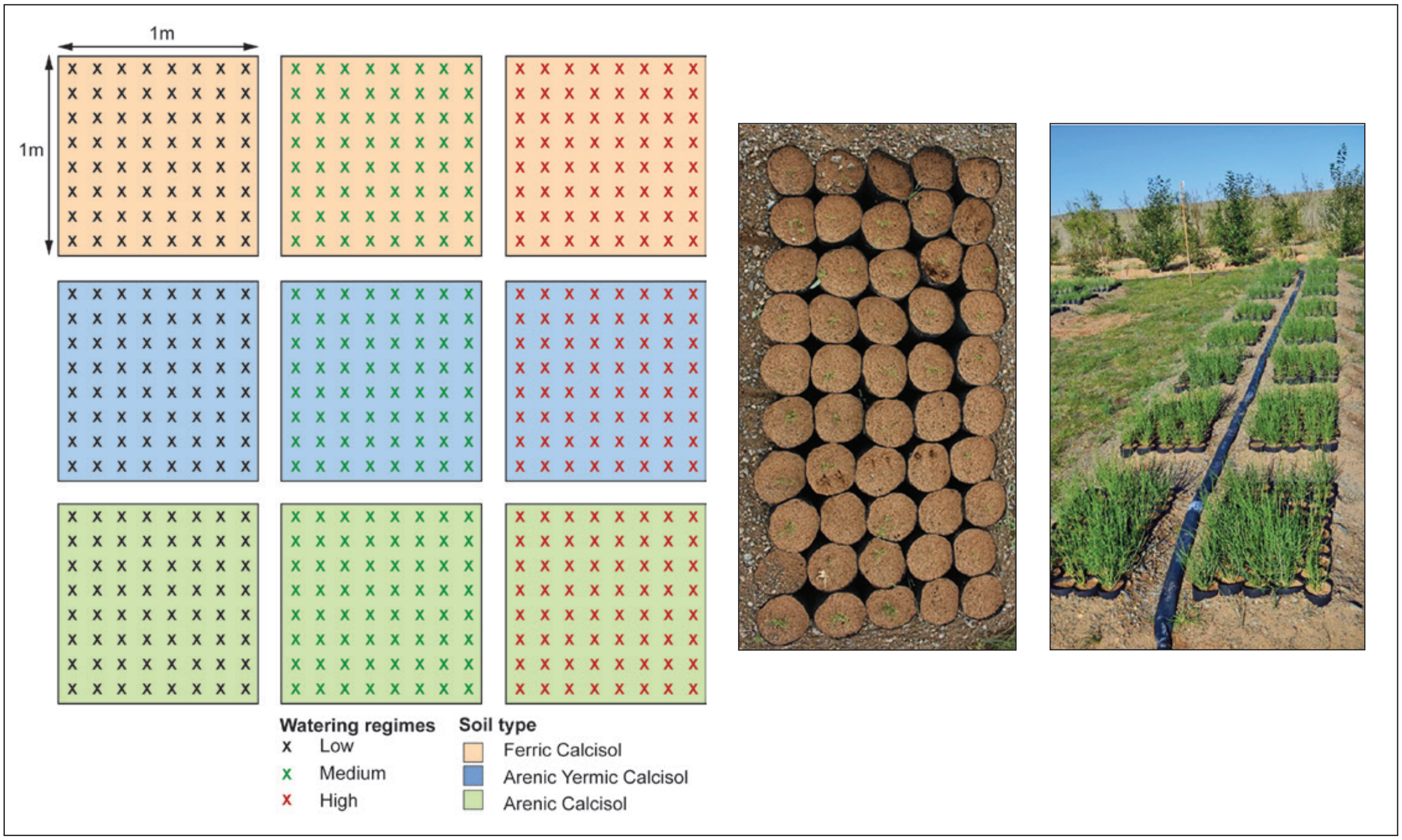

Figure 4. The experimental design for the 3-year study to assess *Haloxylon ammodendron* seedling development included replications of each soil type and watering regime. (Photos by Ser-Oddamba Byambadorj)

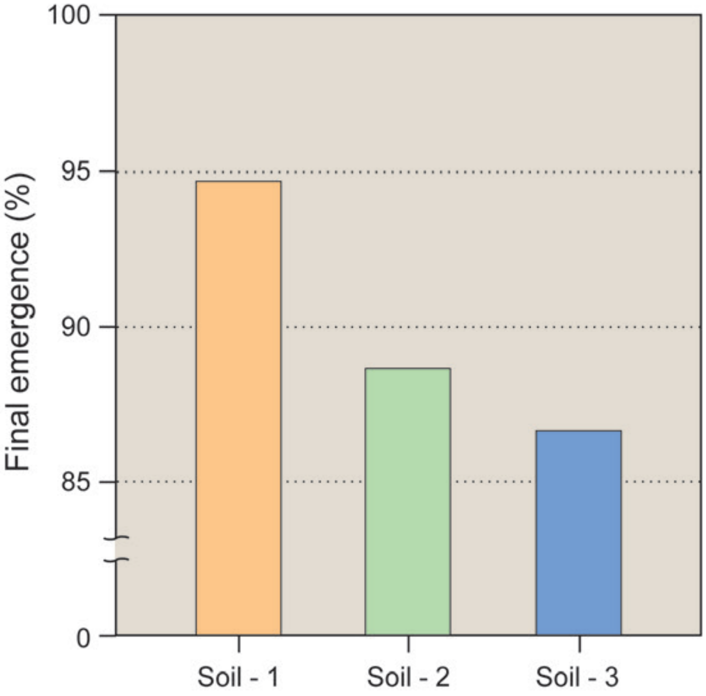

Figure 5. Average total emergence (n=100 containers) of *Haloxylon ammodendron* seeds varied by soil type.

(ANOVA) and Duncan's multiple range test was used for multiple comparisons. Seedling growth and biomass data were normally distributed. ANOVA for the effects of time and treatment on morphological traits was conducted with time and treatment as a fixed effect and plot as a random effect.

Results

Soil Characteristics

Soil physical and chemical characteristics differed among the three soil types (table 1). Soil 1 was the most alkaline with the highest amounts of HCO_3, Cl^-, SO_4^{2-}, Mg^{++}, and Na^+ and, consequently, high EC. Soil 2 had much higher calcium carbonate and organic matter compared with the other two soils. Soil 3 had the highest nitrogen (N), phosphorus (P), and potassium (K) concentrations.

Seed Germination and Emergence

Weight of 1,000 seeds averaged 3.24 ± 0.09 g. The germination capacity and germination energy were 92 and 74 percent, respectively. Seeds sown directly in containers had a lower emergence rate than seeds tested in the laboratory. Nevertheless, after 10 days, emergence was greater than 60 percent regardless of soil type. Final emergence 4 weeks after sowing was more than 85 percent, with Soil 1 having the highest value (figure 5).

Seedling Morphology

In the first growing season, all seedlings initially grew slowly, had accelerated growth in the middle of the season, and grew slowly again toward the end of the season (figure 6). At the low and high watering regimes, seedlings grown in Soil 3 were taller than those grown in the other two soils at the end of the season. In the medium watering regime, seedlings in Soil 1 were tallest. Diameter growth (RCD) followed a similar pattern as height growth. Seedlings growing in Soil 2 had the smallest RCD values regardless of watering regime (figure 6). Height and diameter growth patterns during the second and third growing seasons were similar to the first growing season (figure 7).

At the end of the study (three growing seasons), seedling morphology differed significantly by soil type and watering regime (figure 7 and table 2). Root systems were well developed regardless of treatment (figure 8). Biomass values differed significantly by soil type but not by watering regime (figure 9, table 3). Both root and shoot biomass were largest in seedlings grown in Soil 3 and smallest in seedlings grown in Soil 2.

Table 2. Statistical analysis of height and root collar diameter (RCD) as a function of different treatments at the end of three growing seasons.

	Height			RCD		
Source of variation	DF	F value	Pr > F	DF	F value	Pr > F
Soil type	2	3.18	0.0436	2	7.38	0.0008
Watering regime	2	23.12	<.0001	2	3.98	0.0203
Soil type * watering regime	4	3.70	0.0063	4	0.63	0.6396

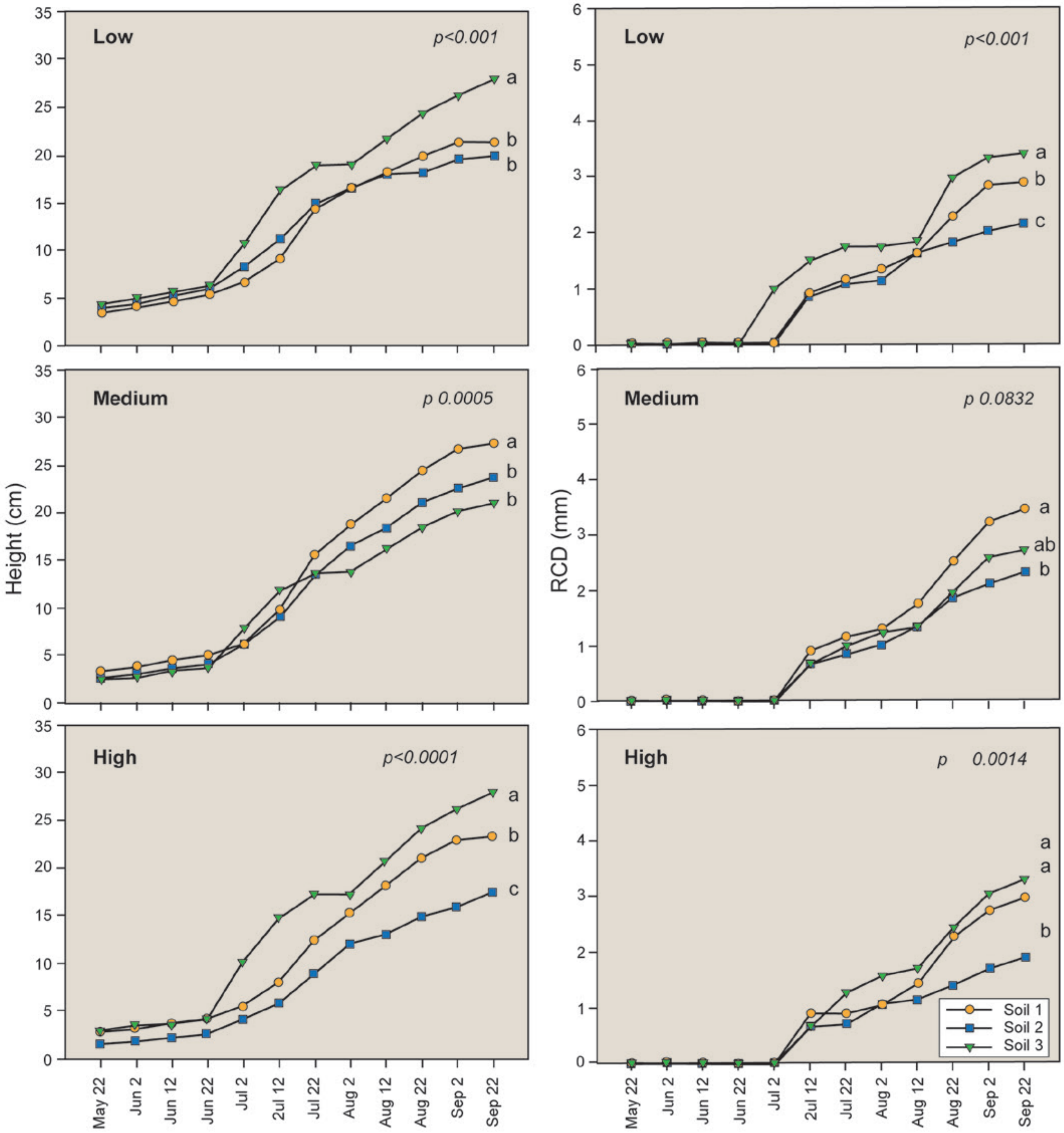

Figure 6. Shoot height and root collar diameter at the end of the first growing season varied (2014) by watering regime (low, medium, and high) and soil type (n = 50).

Discussion

All treatment combinations resulted in good seedling establishment. At the end of the establishment phase, roots were at least 20 to 25 cm long, sufficient for them to find an environment containing more moisture and less salinity than in superficial soil horizons (Matsui et al. 2018, Tobe et al. 2005, Wang et al. 2019). Height and RCD differed among some treatments, but all treatments resulted in a good balance between aboveground and belowground biomass allocation. The mean root-to-shoot ratio after 3 years was approximately 0.8 (data not shown) independent of treatments, considered a well-balanced ratio (Evert 2006).

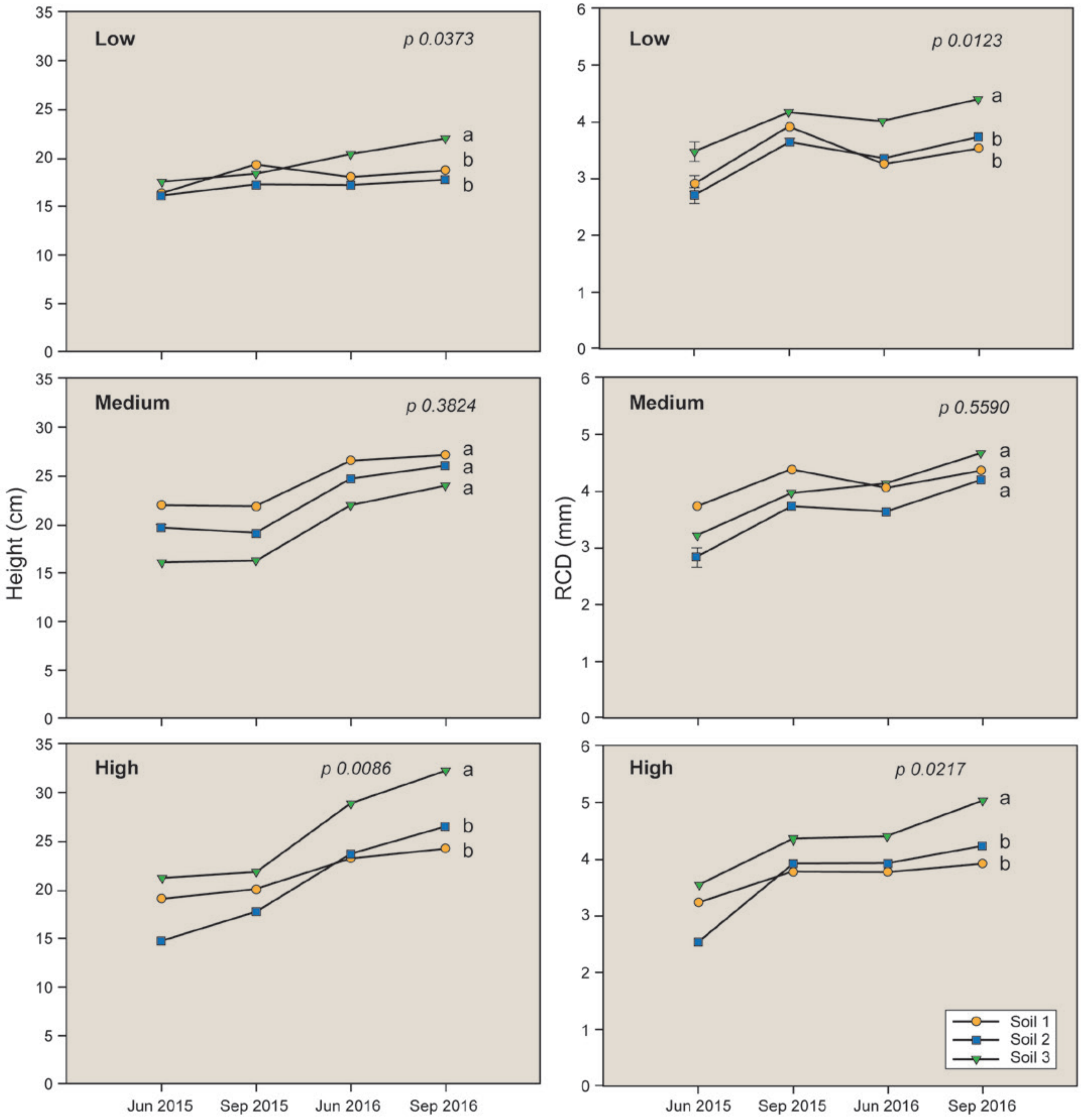

Figure 7. Shoot height and root collar diameter during the second and third growing seasons tended to be highest for Soil 3 (n = 50).

Table 3. Statistical analysis of biomass partitioning as a function of different treatments at the end of three growing seasons.

Source of variation	DF	Root biomass F value	Root biomass Pr > F	Shoot biomass F value	Shoot biomass Pr > F
Soil type	2	10.24	<.0001	7.75	0.0007
Watering regime	2	0.63	0.5358	0.90	0.4078
Soil type * watering regime	4	1.78	0.1369	1.44	0.2239

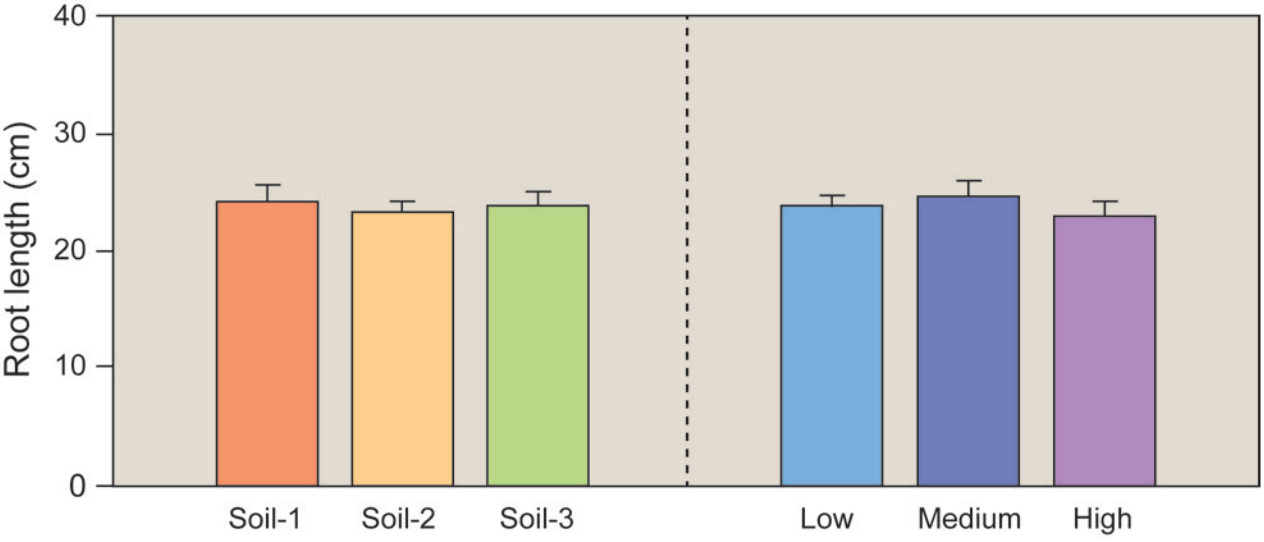

Figure 8. Root length at the end of the third growing season varied little among soil types or watering regime (n = 15).

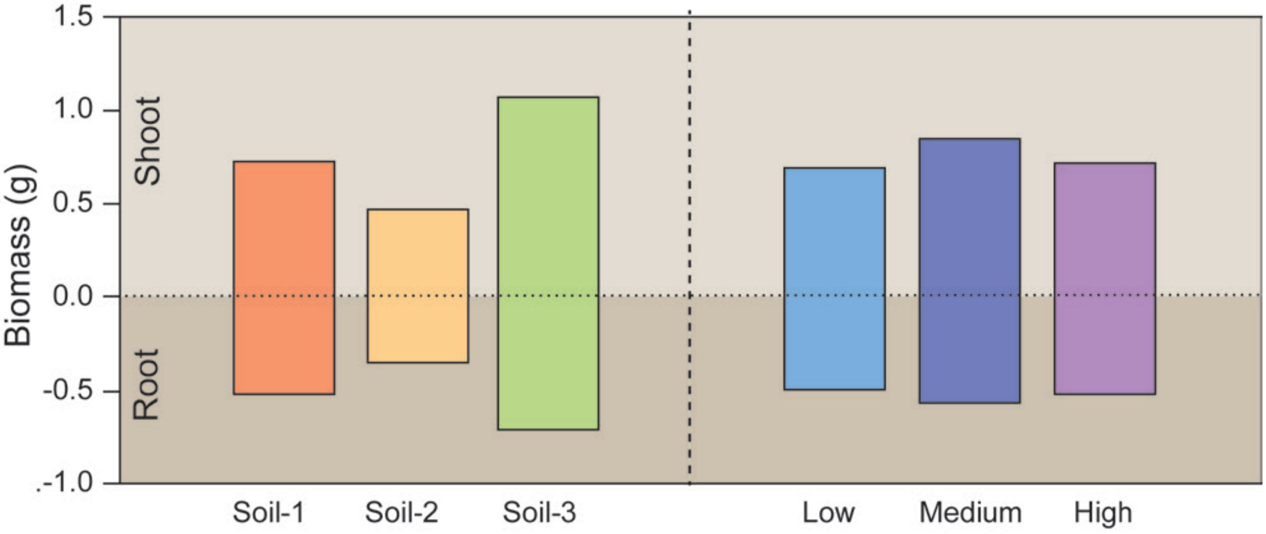

Figure 9. Aboveground and belowground biomass partitioning at the end of the third growing season varied among soil types and watering regime (n = 15).

Height growth slowed during the second season. This growth pattern is similar to results from a previous study with *Haloxylon ammodendron* (Zhang et al. 2016). The authors suggested that a change in biomass partitioning slowed shoot growth and increased root biomass to increase water uptake (Wei et al. 2007). We did not harvest seedlings at the end of the first and second growing seasons to examine biomass partitioning, thus we cannot be certain if this explains the growth patterns in this study. Future investigations could examine the annual pattern of biomass partitioning to better understand how this might represent an adaptation to the arid environment (Wei et al. 2007, Zhu and Jia 2012). Future research could also examine if the use of soil from the designated outplanting site will result in seedlings being preconditioned to their future field environment.

Figure 10. Propagation of *Haloxylon* can be done successfully in a nursery, and could be a significant contributor to restoring this species to the landscape. (Photo by Ser-Oddamba Byambadorj)

Our results highlight the possibility of using soil from the designated outplanting site instead of traditional growing media to produce high-quality *Haloxylon* seedlings in forest nurseries. The most important factor is that the growing medium functions well under the nursery growing conditions (Altmann 2021). Even though the soil used in our study was not sterilized and not modified physically (by sifting) or chemically (by adding compost or other amendments) before using, seedling growth was acceptable, and no disease occurred. Being able to replace traditional growing media may be an economically favorable approach for forest nursery production (though it must be done in an environmentally sustainable manner), particularly when large-scale restoration activities are underway for these arid lands.

Conclusions

Given the likelihood that future climate conditions in central Asia will be hotter and drier, we believe that the focus during the next few decades should center on protecting and arresting further degradation of arid ecosystems. In Mongolia, reforestation and afforestation of arid lands depend upon *Haloxylon* species due to their tolerance of arid conditions. Because natural regeneration of this species is poor, however, propagation protocols in local nurseries are necessary (Khaulenbek et al. 2018). The protocol suggested here demonstrates that high-quality container seedlings can be grown with field soils from the designated outplanting sites using adequate watering regimes (figure 10). Preliminary results for outplanting these seedlings in desert sites near the nursery indicate that 96 to 100 percent survived with slight differences by year-of-planting (Khaider et al. 2019). We will continue long-term evaluation of these afforestation efforts.

Address correspondence to–

John Stanturf, Visiting Professor, Institute of Forestry and Rural Engineering, Estonian University of Life Sciences, Kreutzwaldi 5, Tartu, Estonia, Senior researcher, InNovaSilva, ApS, Højen Tang 80, Vejle, Denmark; email: drdirt48@gmail.com; phone: 585–645–7890 mobile (U.S.)

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