



Assessment of Biomass, Carbon Stock and Rhizospheric Properties of Seabuckthorn shrub (*Hippophae rhamnoides* L.) in Spituk Village of Leh District, Ladakh

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Abstract: Study was carried out at Spituk village of Leh District for assessing the biomass carbon stock and rhizospheric properties of a dioecious shrub-*Hippophae rhamnoides* (Seabuckthorn). Biomass and carbon stock was assessed using the destructive harvest method in 3 quadrat plots of 10 x 10 m each in this study. Soil samples were collected in the quadrat plots from soil underneath Seabuckthorn. It revealed that the stand level total biomass of shrubs attained 30.78 mg ha⁻¹ at diameter class of 6.1-7.0 cm and total stand level vegetation carbon stock also tracked a similar trend to biomass. The total carbon stock in vegetation+soil at stand level was 74.15±8.58 Mg C ha⁻¹. While stand level total nitrogen was 9.80 Mg ha⁻¹ at shallow depth i.e. from 0 to 0.3 m and declines (4.89 Mg ha⁻¹) in the depth range of 0.3-0.6 m. Soil reaction near the shrub indicated alkaline range of pH (8.71±0.24) throughout depth range of (0-0.9 m) with electrical conductivity in the upper top layer (0-0.3 m) higher as compared to deeper zone (0.3-0.9 m). It is concluded that *Hippophae* should be considered as potential shrub for carbon stock development reserve and soil improvement in Ladakh.

Key words: Seabuckthorn, cold desert Ladakh, biomass, carbon stock, nitrogen.

Seabuckthorn (*Hippophae rhamnoides*) Eleagnaceae shrub, spread across temperate regions of Europe and Asia is a native to the Trans-Himalayan Ladakh region. It is a dioecious, deciduous, wind pollinated, spinescent shrub bearing red, orange and yellow berries which have medicinal and economic importance. It is distributed to an area of 13000ha in Ladakh region (Stobdan *et al.*, 2017). In addition, it is a drought tolerant and grows in extreme temperate ranges (-40 to 40°C) and accumulates carbon reserves to mitigate climate change. It is also a nitrogen-fixing actinorhizal shrub reported to fix nitrogen in association with *Frankia*-a nitrogen fixing bacteria residing in its roots (Kato *et al.*, 2007). Its forest with increasing age also enhanced the total soil organic carbon and nitrogen in soil and maintained alkaline soil pH (Yang *et al.*, 2018). Biomass is the direct reserve of carbon stock in vegetation. *Hippophae rhamnoides* compared to the shrubs: *Haloxylon ammodendron*, *Vitex negundo* var. *heterophylla* and *Caragana* spp. in the three north shelterbelt forest region of China have the highest mean biomass density, carbon density, and carbon sequestration rate (Wenhui *et al.*, 2014). In this age of global

warming and climate change. United Nations Climate Change Convention has desired to restrict global average temperature rise below 2°C and limit the temperature rise to 1.5°C above pre-industrial levels (UNFCCC, 2015). Under the present situation cold-desert Ladakh is most vulnerable due to its unique ecosystem comprising Trans-Himalayan, high altitude, low precipitation, extreme temperatures and remote location. It is suggested to plant *Hippophae rhamnoides*, willow and poplar along the Shyok and Nubra river belt in Ladakh for carbon sequestration (Kumar *et al.*, 2009). Seabuckthorn is designated as bio-resource and its medicinal and economic importance has been extensively studied in Ladakh (Stobdan *et al.*, 2011; Stobdan *et al.*, 2015) but community studies on its ecological benefits in Ladakh is lacking. Extensive research on ecological benefits of Seabuckthorn in China has demonstrated the potential of this shrub to improve carbon stock and soil nutrient status. Ladakh is a fragile environment owing to its cold desert high altitude, scanty vegetation and oligotrophic soils and it is vulnerable to changing climate. Biomass in vegetation is a long term reserve of carbon. The plants assimilate atmospheric carbon in biomass

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through the process of photosynthesis. Carbon is also assimilated by plant roots from the soil. Carbon content of plant biomass is globally estimated to be in a range of 45.01 to 47.88% (Ma *et al.*, 2018). Therefore, biomass and carbon stock in plants are directly proportional and related to each other. Keeping all in this in view, it was designed to study the potential of *Hippophae rhamnoides* as an ecological resource and assessing its biomass, carbon stock in biomass, soil organic carbon, total nitrogen, pH and electrical conductivity in soil.

Materials and Methods

Experimental setup

The study was carried out at DRDO-Defence Institute of High Altitude Research (DIHAR), Leh-Ladakh and Nalanda University, Rajgir, Bihar during June 2017-April 2018 of naturally occurring Seabuckthorn shrub in two stages. First stage comprised of randomly selected 8 *Hippophae rhamnoides* shrubs of age 2.5 years in two diameter classes (0.1-1.0 cm) and (1.1-2.0 cm) at DRDO-DIHAR, Leh-Ladakh. While in second stage, shrub ages ranged from 6 years to 22 years in the diameter classes (4.1-5.0 cm), (5.1-6.0 cm) and (6.1-7.0 cm) at Spituk village of Leh district near Indus belt (34°7'4.54"N, 77°32'1.64"E) (Fig. 1). The mean annual precipitation is lower than 100 mm mostly in the form of snowfall (Shafiq *et al.*, 2016). During summer, the maximum temperature recorded was +34.8°C and minimum temperature -27.9°C i.e. during winter (Chevuturi *et al.*, 2016). The soil type is entisol (Gupta and Arora, 2017).

Biomass assessment

Seabuckthorn (*Hippophae rhamnoides*) shrub (age: 2.5 years) in diameter classes (0.1 to 1.0 cm) and (1.1 to 2.0 cm) were randomly harvested using destructive harvest method from DIHAR experimental plot for stage-1 for comparative purpose and for determining root: shoot (R: S) ratio which is a ratio of below ground (root) biomass to above ground (shoot) biomass in plants. The age was considered since the time of plantation. The diameter of individual shrubs at breast height was recorded using vernier caliper and computed into diameter classes. The above-ground (stemwood, branchwood, and leaves and foliage) and below ground portion (root) of shrubs were included in the

study. The soil on below ground part (roots) was comprehensively cleared and the fresh weight of the below-ground part was recorded.

In stage-2, a total of 30 *Hippophae rhamnoides* shrubs were destructively harvested using quadrats randomly. Ten plants per plot were harvested in three plots of 10 m × 10 m each during the month of June-July 2017. Three sample wooden discs were collected from each of the above and below ground parts. Annual rings were counted to determine the age of shrub. The age ranged from 6 to 22 years. Their diameter at breast height was recorded as in stage-1. Dry biomass of various above and below ground components was calculated from the moisture content of samples and fresh weights of their source tissue. The dry biomass of shrub components was summated to derive the total biomass of each shrub. The diameter class wise roots to shoot ratio and mean height were also estimated. The carbon content of biomass is reported to be in a range of 45-50% (Schlesinger, 1991). In this study the equation used to calculate carbon content of biomass is Carbon content = 47.5% of oven dry biomass. This avoids the upper and lower extremes of 45 and 50% respectively for minimizing errors. The carbon content of each shrub component was aggregated to determine carbon content of full shrub. The average shrub biomass and carbon stock were calculated and multiplied by the total number of trees in one hectare to determine biomass and carbon stock in one hectare.

Soil characteristic assessment

Soil profile cores of 0.9 m depth was made using soil corer for determination of total soil organic carbon, total soil nitrogen, pH and electrical conductivity in the rhizosphere soil under Seabuckthorn-*Hippophae rhamnoides* and under grassland adjacent to *Hippophae rhamnoides* plots (for comparison) at three depth ranges (0 to 0.3 m), (0.3 m to 0.6 m) and (0.6 m to 0.9 m). The analysis of total soil organic carbon and total soil nitrogen was done using Shimadzu TOC/L analyzer instrument model TOC-L CPN. pH was determined using pH meter instrument, HACH-Sension + pH-meter. Electrical conductivity was determined using EC meter instrument, HACH-Sension + EC 71 meter.

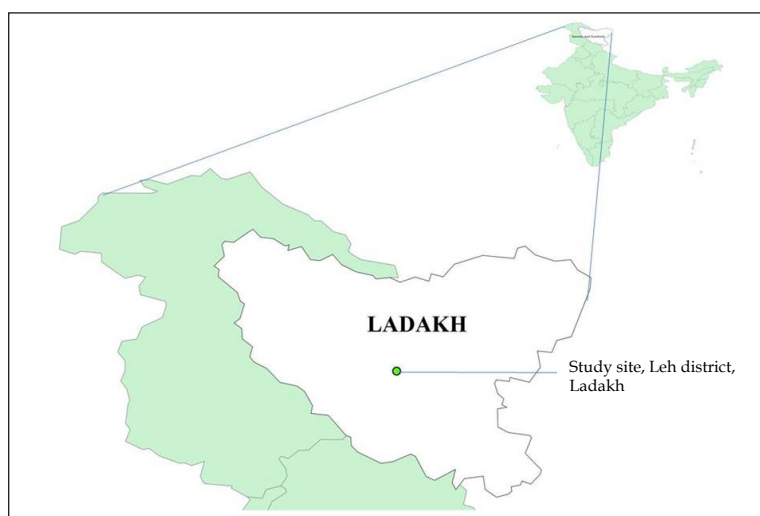


Fig. 1. Ladakh region is highlighted in Indian map developed by ArcMap software and study site is represented in Leh District.

Data analysis

The data for biomass and vegetation carbon stock samples were analyzed with t-Test between means of each diameter class. The means of soil sample parameters, total soil organic carbon, total soil nitrogen, pH and electrical conductivity estimated under Seabuckthorn (*Hippophae rhamnoides*) and grassland were compared using t-test between respective ranges.

Results and Discussion

The first stage of the study was done in DIHAR, Leh and it comprised of 2.5 year old 8 Seabuckthorn-*Hippophae rhamnoides* shrubs. The first stage study was done as an initial study to determine the biomass, carbon stock and root to shoot ratio of the shrub. These parameters of Seabuckthorn were unknown for the Ladakh region and therefore, the results of stage one gave clear insights to carry this study further on a larger scale in naturally growing Seabuckthorn stand. After completing stage one, a ground survey was carried out in Leh district to select naturally growing Seabuckthorn-*Hippophae rhamnoides* stand and the study site was decided and selected in the Spituk village of Leh district. This site comprised naturally growing stand of Seabuckthorn-*Hippophae rhamnoides* which were in diverse range of diameter classes and ages of the shrub. Since this study was done by destructive harvest, therefore to minimize clearing of shrubs a minimum of 3 quadrat plots of 10 m x 10 m were used to harvest a

total of 30 shrubs, 10 shrubs from each quadrat. This sampling intensity was required to have a statistically significant sample to minimize the possibility of errors in the analysis of the results.

Biomass stock

Biomass stock is the total amount of plant matter present in plant at any specific time. It is a long term reserve for sequestering carbon in plants through the process of photosynthesis. Biomass can be used to determine carbon content in plants, and the allocation of plant matter above ground in shoots and in below ground roots. During this study in Stage-1: The aboveground and belowground biomass of Seabuckthorn (*Hippophae rhamnoides*) shrubs among the two diameter classes (0.1-1.0 cm) and (1.1-2.0 cm) were statistically similar among stage-1 ($p < 0.05$) and statistically different from stage-2 ($p < 0.05$) in diameter class 4.1-5.0 cm, 5.1-6.0 cm and 6.1-7.0 cm (Table 1). The above ground biomass stock ranged from 67.54% in diameter class from 6.1-7.0 cm to 70.55% in 5.1-6.0 cm and below ground biomass stock ranged from 29.41% in 5.1-6.0 cm to 32.42% in 6.1-7.0 cm in the stage-2 (Table 1). Zhang and Chen, 2007 also reported that above ground biomass of *Hippophae rhamnoides* around 76.01% and below ground biomass nearly around 23.99% in its pure stand in eastern Loess plateau of China which is similar to the findings of this study (Table 1). Higher below ground biomass stock (root) in this study compared to (Zhang and Chen, 2007) can be attributed to the stressful

Table 1. Biomass production of Seabuckthorn (*Hippophae rhamnoides*) shrubs

	Diameter class	Aboveground		Belowground		Total	
		Per shrub (kg shrub ⁻¹)	Stand (Mg ha ⁻¹)	Per shrub (kg shrub ⁻¹)	Stand (Mg ha ⁻¹)	Per shrub (kg shrub ⁻¹)	Stand (Mg ha ⁻¹)
S(1)	0.1-1.0, n=5	0.02 ^a (0.008)	0.06 ^a (0.02)	0.01 ^a (0.008)	0.04 ^a (0.02)	0.03 ^a (0.01)	0.11 ^a (0.05)
	1.1-2.0, n=3	0.24 ^a (0.30)	0.79 ^a (0.99)	0.19 ^a (0.29)	0.64 ^a (0.96)	0.43 ^a (0.59)	1.43 ^a (1.95)
S(2)	4.1-5.0, n=14	4.87 ^b (1.87)	16.09 ^b (6.17)	2.13 ^b (0.78)	7.04 ^a (2.59)	7.01 ^b (2.63)	23.13 ^b (8.71)
	5.1-6.0, n=10	6.16 ^b (1.37)	20.34 ^b (45.35)	2.57 ^b (0.61)	8.48 ^b (2.03)	8.73 ^b (1.90)	28.83 ^b (6.57)
	6.1-7.0, n=6	6.30 ^b (1.13)	20.79 ^b (3.72)	3.02 ^b (1.45)	9.98 ^a (4.79)	9.32 ^b (2.27)	30.78 ^b (7.50)

S: Stage

n: Number of *Hippophae rhamnoides* shrubs in each respective diameter class

Values in parenthesis are standard deviation of means; values with the same superscripts within columns do not differ significantly at 0.05 level.

high-altitude cold-desert environment of Ladakh with low precipitation and low oxygen levels.

Root to shoot ratio (stage 1 and 2)

Root to shoot ratio is useful in studies where one of the two components in biomass: above ground or below ground is unknown and using root to shoot ratio it could be determined. Root to shoot ratio of Seabuckthorn in Ladakh has not been studied before. Therefore the results of the present study would avoid further destructive harvest of this shrub for future studies in Ladakh. During this study in stage-1, the total below ground biomass stock was in the range of 36.36 to 44.75% and Root: shoot ratio 0.75 to 0.52 in diameter class 0-0.1 cm to 1.1-2.0 cm, respectively (Fig. 2).

While in stage-2, the root to shoot ratio ranged from 0.41 to 0.47 in the diameter classes 5.1-6.0 cm to 6.1-7.0 cm, between the age group

of 6-22 years (Fig. 2). Findings of root to shoot ratio of shrubs in this study is in the range of 0.43 to 1.07 of the shrubs reported also by (Li *et al.*, 2004). Further, it is clearly indicated that allocation of below ground biomass is reduced as the stage advances.

Biomass carbon stock (stage-2)

Biomass carbon stock is a direct reserve of carbon in biomass of plants. It determines the capacity to the plant to sequester carbon, carbon flux, and its long term storage in the plant biomass. In this study on Seabuckthorn the total mean per shrub carbon stocks in the diameter classes (4.1-5.0 cm), (5.1-6.0 cm) and (6.1-7.0 cm) were significantly similar ($p < 0.05$) and it ranged from 3.33 kg C shrub⁻¹ in the diameter class (4.1- 5.0 cm) to 4.43 kg C shrub⁻¹ in the diameter class (6.1-7.0 cm) (Table 2). The above ground carbon stock per shrub was higher compared to the below ground (roots) in all three diameter classes studied (Table 2).

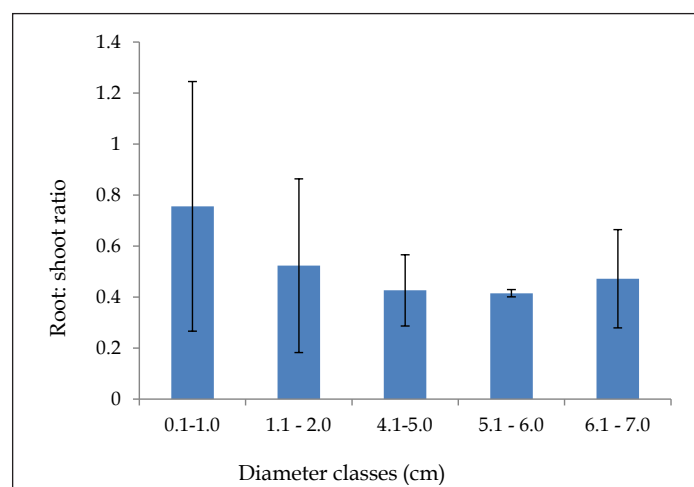


Fig. 2. Root: Shoot (RS ratio) of Seabuckthorn-*Hippophae rhamnoides* in diameter classes 0.1-1.0 cm, 1.1-2.0 cm, 4.1-5.0 cm, 5.1-6.0 cm and 6.1-7.0 cm.

Table 2. Carbon stocks in above and below ground biomass fractions of Seabuckthorn (*Hippophae rhamnoides*) shrubs across diameter classes (4.1-5.0), (5.1-6.0) and (6.1-7.0) cm in Spituk, Leh, Ladakh, India

Diameter class	Aboveground		Belowground		Total	
	Per shrub (kg C shrub ⁻¹)	Stand (Mg C ha ⁻¹)	Per shrub (kg C shrub ⁻¹)	Stand (Mg C ha ⁻¹)	Per shrub (kg C shrub ⁻¹)	Stand (Mg C ha ⁻¹)
S(2) 4.1-5.0, n=14	2.31 ^b (0.88)	7.64 ^b (2.93)	1.01 ^b (0.36)	3.34 ^b (1.19)	3.33 ^b (0.95)	10.99 ^b (3.16)
5.1-6.0, n=10	2.92 ^b (0.65)	9.66 ^b (2.15)	1.22 ^b (0.29)	4.03 ^b (0.96)	4.14 ^b (0.71)	13.69 ^b (2.36)
6.1-7.0, n=6	2.99 ^b (0.53)	9.87 ^b (1.77)	1.43 ^b (0.68)	4.74 ^b (2.37)	4.43 ^b (0.87)	14.62 ^b (2.88)

S: Stage

n: Number of *Hippophae rhamnoides* shrubs in each diameter class

Values in parenthesis are standard deviation of means; values with the same superscripts within columns do not differ significantly at 0.05 level.

The above ground mean carbon stock per shrub ranged from 67.49% in the diameter class (6.1-7.0 cm) to 70.53% in the diameter class (5.1-6.0 cm) (Table 2). The mean carbon stock accounted by below ground part (root) ranged from 29.46% in the diameter class (5.1-6.0 cm) to 32.27% in the diameter class (6.1-7.0 cm). The stand level carbon stock had a similar trend to the mean shrub carbon stock (Table 2). Total stand level carbon stocks in the diameter classes (4.1-5.0 cm), (5.1-6.0 cm) and (6.1-7.0 cm) were significantly similar ($p < 0.05$) (Table 2).

The stand level soil carbon stock is 4.92 times higher than the vegetation carbon stock (Table 3). The key advantage of growing Seabuckthorn shrub in cold-desert is the production of carbon reserve in biomass and soil. The carbon stock in biomass of Seabuckthorn shrubs determined in this study is 12.52 Mg C ha⁻¹ and is lower than the carbon stock of 16.5 Mg C ha⁻¹ in its stand reported by Wenhui *et al.*, 2014 in their study at the Loess plateau sub region of China. This difference can be attributed to the differences in annual temperature, precipitation, elevation and soil type of Ladakh and Loess plateau region.

Carbon stock of this shrub is also an indicative to its carbon sequestration capacity. Therefore, Seabuckthorn along with willow and poplar is recommended for production for high carbon stock at Ladakh (Kumar *et al.*, 2009).

Total soil organic carbon and soil total nitrogen stock

Soil organic carbon contributes to carbon sequestration, plant uptake of carbon, nutrient retention and exchange, maintenance of soil structure and regulator of soil moisture. Total nitrogen is an indicator of soil quality and it is strongly correlated with soil organic carbon. Soil organic carbon and soil total nitrogen are essentials of pedogenic processes and are contributors to soil fertility. The results in this study could be set as the baseline to start the further monitoring of the soil organic carbon and nitrogen fluxes between Seabuckthorn and soil in Ladakh. Total soil organic carbon and total nitrogen stocks varied significantly with varied depth ranges till 0.9 m soil depth (Table 4). Although the total carbon and nitrogen in grassland compared to Seabuckthorn was higher in the (0-0.3 m) and (0.3-0.6 m) depth range (Table 4). Highest levels of total organic carbon and total nitrogen in soil under Seabuckthorn and grassland in 0-0.3 m depth (Table 4) further indicates that upper soil layer has more accumulation of the nutrients, also supporting other vegetation in integrated manner. Results are in confirmatory to the studies carried out by Acharya *et al.* (2012) in Ladakh. This is attributed to high levels of litter fall and organic matter decomposition on the top soil layer in grassland compared to this shrub, though a comprehensive study is required to understand the exact cause of

Table 3. Total, above and below ground carbon stocks on a stand level for Seabuckthorn (*Hippophae rhamnoides*) shrubs across all diameter classes of Stage-2 in Spituk, Leh, Ladakh, India

Above ground vegetation (Mg ha ⁻¹)	Root (Mg ha ⁻¹)	Total (Above ground + root) (Mg ha ⁻¹)	Soil (Mg ha ⁻¹)	Total (Vegetation +soil) (Mg ha ⁻¹)
8.69 (2.66)	3.83 (1.48)	12.52 (3.05)	61.63 (8.02)	74.15 (8.58)

Values in parenthesis are standard deviation of means.

Table 4. Soil chemical properties (total soil organic carbon and total soil nitrogen) under *Hippophae rhamnoides* and grassland

Depth range (m)	Total soil organic carbon Mg ha ⁻¹		Total soil nitrogen Mg ha ⁻¹	
	Seabuckthorn ¹	Grassland	Seabuckthorn ¹	Grassland
0 to 0.3	26.96 ^{aA} (7.63)	28.90 ^{aA} (6.52)	9.80 ^{aA} (5.13)	10.68 ^{aA} (2.17)
0.3 to 0.6	16.61 ^{aA} (2.12)	23.33 ^{aB} (0.13)	4.89 ^{aA} (1.88)	8.12 ^{aB} (0.82)
0.6 to 0.9	18.06 ^{aA} (1.25)	14.63 ^{bB} (1.28)	5.18 ^{aA} (1.89)	4.94 ^{aB} (1.10)
(Total) 0 to 0.9	20.54 ^A (6.29)	22.29 ^B (7.06)	6.62 ^A (3.75)	7.91 ^A (2.80)

¹Seabuckthorn: *Hippophae rhamnoides*

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this difference. The deepest depth range (0.6-0.9 m) recorded higher total organic carbon and total nitrogen under Seabuckthorn plots compared to the Grassland plots (Table 4). The decomposition of Seabuckthorn roots and nitrogen fixation in soil by its root nodules in deeper layers may have contributed to the total soil organic carbon and total nitrogen in the (0.6-0.9 m) depth range which resulted in higher total organic carbon and total nitrogen in Seabuckthorn soil compared to the grassland soil (Table 4). It could be explained by spread of Seabuckthorn roots which were present throughout the sampled soil depth of 0.9 m enabling root decomposition in deeper layers. Seabuckthorn roots have been reported penetrate soil to a depth range of 3 to 5 m (Chengjiang and Diaquiong, 2002). The entire studied soil depth of (0.9) m witnessed higher total soil organic carbon and total soil nitrogen in grassland soil compared to Seabuckthorn soil (Table 4). Seabuckthorn growth on the site soil can be considered as an indicator of its potential to sequester carbon and nitrogen fixation in soil. However, temporal study of this aspect shall further strong then our conclusion with respect to carbon and nitrogen stock.

pH and electrical conductivity

Seabuckthorn and grassland plots recorded similar pH upto 0.9 m depth. While electrical conductivity of soil is higher under Seabuckthorn plot as compared to grassland upto 0.9 m depth (Table 5). Sharma and Singh, 2017 also reported that the pH of soil is alkaline under *Hippophae* stand in dry temperate region. Further, presence of calcerous parent material in cold desert at high altitude soil profile renders the pH alkaline (Charan *et al.*, 2013). The electrical conductivity is highest in the upper layer (0-0.3 m) of soil under Seabuckthorn stand compared to grassland which could be explained by high organic matter deposition on the top soil layer by Seabuckthorn shrubs (Table 5). The electrical conductivity determined in the present study is similar to that by Charan *et al.*, 2013 who reported the EC of Ladakh soils in their study to be $294.72 \pm 21.85 \mu\text{S cm}^{-1}$ in a range of altitude 11000 to 12000 feet amsl. Our study site lies in this range in the current study and is located at 11500 feet amsl.

Conclusion and Suggestions

This study is very useful in generating baseline data on biomass, carbon stock, total

Table 5. Electrical conductivity and pH under *Hippophae rhamnoides* (Seabuckthorn) and grassland

Depth range (m)	pH		Electrical conductivity $\mu\text{S m}^{-1}$	
	Seabuckthorn ¹	Grassland	Seabuckthorn ¹	Grassland
0 to 0.3	9.03 ^{aA} (0.03)	8.93 ^{aB} (0.01)	706.00 ^{aA} (5.56)	215.66 ^{aB} (3.05)
0.3 to 0.6	8.55 ^{bA} (0.01)	8.73 ^{bB} (0.02)	352.66 ^{bA} (2.30)	202.33 ^{bB} (3.05)
0.6 to 0.9	8.54 ^{bA} (0.04)	8.84 ^{cB} (0.02)	344.66 ^{cA} (3.21)	256.33 ^{cB} (2.51)
(Total) 0 to 0.9	8.71 ^A (0.24)	8.83 ^A (0.08)	470.44 ^A (176.69)	206.77 ^B (7.17)

¹Seabuckthorn: *Hippophae rhamnoides*

Values in parenthesis are standard deviation of means; values with the same superscripts in lower case letters within columns do not differ significantly for each parameter. Values with the same superscripts in upper case letters within rows do not differ significantly for each parameter at 0.05 level.

soil nitrogen and changes in pH and electrical conductivity with respect to Seabuckthorn (*Hippophae rhamnoides*) in Ladakh. It is indicated in this study that Seabuckthorn shrubs should be considered as a potential shrub to sequester carbon in biomass and soil, fixing nitrogen in soil and improving soil fertility. Its plantation in cold-desert high altitude environment of Ladakh has ecological benefits and could be a sustainable strategy for development of carbon stock reserves in vegetation and soil. Ladakh's environment is fragile and vulnerable to climate change in this current era of global warming owing to its high altitude cold desert features. Therefore, plantations would be the best mitigation strategy for climate change impacts in this region. Since this study was a preliminary research to assess the ecological benefits of Seabuckthorn shrubs in Ladakh region therefore to proceed further it is suggested that a time sequence-based study need to be conducted on Seabuckthorn shrub carbon sequestration and its impact on soil nutrient and chemical properties. A comprehensive study on mixed plantations of Seabuckthorn with willow and poplar is needed to assess its synergistic impact on biomass and carbon stock production in the Ladakh region as the other two are also prevalent species in the region.

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