Nitrogen-fixing potential of *Halimodendron halodendron* (Pall.) Voss in arid and semi-arid areas of Iran

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Abstract

Nitrogen content was estimated in rhizosphere of legume *Halimodendron halodendron* (Pall.) Voss along with *Berberis integerrima* Bunge as control in sandy loam soils of arid and semi-arid areas in Middle East every 2 years (2013–2019). *Lepidium sativum* seed was also cultivated in soil of shrubs rhizosphere for monitoring its performance and N content. Maximum rates of 1.05% year⁻¹ and 1.54% year⁻¹ for soil organic nitrogen (ON) and 0.50% year⁻¹ and 0.66% year⁻¹ for mineral nitrogen (MN) were observed in *H. halodendron* rhizospheres in arid and semi-arid areas, respectively. Moreover, the highest *L. sativum* performance occurred in *H. halodendron* rhizosphere. Significant relation also was observed between N content of *L. sativum* and N content of shrubs rhizosphere ($p < 0.02$; $R^2 = 0.606$ and 0.585 between SON and MN of soil, respectively, and *L. sativum* N content).

Keywords Arid and semi-arid conditions · Nitrogen fixation · Rhizosphere · Vegetative characteristics

1 Introduction

Arid and semi-arid areas cover more than 40% of Earth's terrestrial surface [1, 2]. These lands have high potential in terms of capturing and fixing atmospheric nitrogen (N). In these areas, soil N balance assessment is a useful tool for estimating the magnitude of N management [3, 4] to facilitate the restoration ecology [5, 6].

In arid lands, however, long-term ecological restoration improves the potential nitrogen fixation (NF) [7] and fixing N plants have special importance [8]. Under arid and semi-arid conditions, potential of biological nitrogen fixation (BNF) varies based on plant species and environmental conditions [9–12]. Therefore, introducing plant species, i.e., woody perennial plants [13] with high potential has long been considered for increasing soil N storage in these natural terrestrial ecosystems [12, 14].

BNF is a potentially attractive alternative source of N in ecosystems which is an energetically expensive [15] and

sensitive process that influenced by nutrient and environmental conditions [16]. Generally, legumes are very important both ecologically and agriculturally because they are responsible for a substantial part of the global flux of nitrogen from atmospheric N to fixed forms such as ammonia, nitrate, and organic nitrogen [17]. Moreover, legumes are an important component of the arid and semi-arid environment contributing to the soil fertility through their N fixing activity [18]. In these areas, resistant and durable shrubs/trees play a vital role in ecosystem functions and N turnover and availability [19]. Therefore, it is important to identify plant species with high NF potential that are ecologically adapted to these harsh conditions [5, 20]. However, the reports from NF in plants showed contrasting results and NF potential should be investigated for each specie considering its environmental conditions.

Halimodendron halodendron is a keystone legume species covering a wide area of the dry ecosystems in the Middle East, but no data are available about its NF potential.

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Therefore, this study focused on the NF potential of this species along with a non-legume *Berberis integerrima* in arid and semi-arid ecosystems of Mute and Ardeste, in Iran through 7 years. In addition, we measured biomass production and N content of a tested plant (*Lepidium sativum*) cultivated in soil of *H. halodendron* and *B. integerrima* rhizosphere.

2 Materials and methods

2.1 Study area

Two study sites, Mute in arid and Ardeste in semi-arid lands in Iran were selected for the assessment. These areas have covered mainly with two keystone shrubs of *H. halodendron* and *B. integerrima*. Mute region (Site 1; 50° 13'–51° 02' 00" E, 33° 23'–34° 01' 00" N) covers an area of approximately 214,000 ha in the northwest of Meymeh and southwest of Delijan (Isfahan Province, Iran). The average elevation of the area is 2500 m above sea level. Based on Domarten's classification method, the climate of the region is dry. The area has an annual rainfall of 236 mm and an average annual temperature of 13.5° C.

Ardeste forest reservoir (Site 2; 55° 67' 00"–56° 03' 00" E, 35° 30' 80"–35° 32' 10" N) covers approximately 250 ha located at 11 km from Dehaghan (a city in Isfahan Province). This reservoir was constructed in 1996 with Barberry (*Berberis integerrima* Bunge), Hackberry tree (*Celtis caucasica* Willd.), Hawthorn (*Crataegus persica* A. Pojark.), Pistachios (*Pistacia atlantica* Desf.), and a range of plant species such as *Bromus tectorum* L., *Artemisia aucheri* Boiss., *Astragalus* spp, *Eryngium billardieri*, and *F. Delaroche*. The mean elevation of the area is 2322 m above sea level. The area has an annual rainfall of 347 mm and an average annual temperature of 14.22° C. Based on Domarten's classification method, the region has a semi-arid climate.

2.2 Plant species

Halimodendron halodendron (Pall.) Voss and *Berberis integerrima* Bunge are two dominant plant species covering a wide area of the dry ecosystems in the Middle East. These species create a shelter for fauna, improve environmental conditions, supply forage, and conserve and enrich the soil and flora of these areas. *H. halodendron* is a species of legume native to North Asia and Iran. It is a wild deciduous flowering spiny shrub growing to 2 m in height [21]. *B. integerrima* is a spiny shrub that grows to a height of 1–3 m and is found throughout the temperate and subtropical regions of the world [22].

2.3 Data collection

2.3.1 Soil and vegetation studies

Soil and vegetation cover characteristic were observed based on randomly-systematic method using permanent plots every two years during 2013–2019. Forty-eight soil profiles (0–20 and 20–40 cm soil depth) were excavated using systematic random sampling [23] from one m² quadrates spaced at 50-m intervals along three randomly placed 300 m transects. Soil samples were collected from the root zones of *H. halodendron*, *B. integerrima*, and bare areas between plants to determine the NF potential of the shrubs. The soils were tested for organic nitrogen (ON) and mineral nitrogen (MN) at the Soil Laboratory of Isfahan University of Technology (Isfahan, Iran).

Also, other soil characteristics of acidity (pH), electrical conductivity (EC), organic carbon (OC), phosphorous (P), potassium (K), calcium carbonate (CaCO₃), and texture (clay, silt, sand, gravel percentage) were measured (Table 1).

Vegetative characteristics of two species also were recorded based on the placed plots. Height (cm/plot),

Table 1 Physiochemical characteristics of soil in the study area

	Soil depth	Mute	Ardeste
Gravel _{>2 cm} (%)	d ₁ *	39.70 ± 7.22 ^A	37.50 ± 6.95 ^{Ans}
	d ₂	45.50 ± 7.40 ^A	48.67 ± 8.15 ^{Ans}
Sand (%)	d ₁	58.95 ± 8.05 ^B	31.80 ± 3.25 ^{A**}
	d ₂	65.96 ± 9.40 ^A	73.80 ± 12.64 ^{Ans}
Silt (%)	d ₁	21.19 ± 4.42 ^B	32.60 ± 9.13 ^{A*}
	d ₂	19.70 ± 4.70 ^A	13.08 ± 7.55 ^{B*}
Clay (%)	d ₁	19.68 ± 4.22 ^B	35.66 ± 6.25 ^{A**}
	d ₂	14.33 ± 7.02 ^A	13.15 ± 2.64 ^{Ans}
pH	d ₁	8.55 ± 0.40 ^A	7.13 ± 0.15 ^{B*}
	d ₂	8.21 ± 0.24 ^A	8.00 ± 0.10 ^{Ans}
EC (ds/m)	d ₁	1.12 ± 0.12 ^A	0.78 ± 0.10 ^{B*}
	d ₂	0.86 ± 0.10 ^A	0.49 ± 0.03 ^{B**}
P (Mg/Kg)	d ₁	32.61 ± 3.12 ^A	29.67 ± 5.22 ^{Ans}
	d ₂	36.01 ± 4.03 ^A	21.22 ± 3.13 ^{B**}
CaCO ₃ (%)	d ₁	18.67 ± 4.42 ^A	16.33 ± 5.41 ^{Ans}
	d ₂	23.40 ± 5.32 ^A	22.22 ± 6.32 ^{Ans}
CaSO ₄ (%)	d ₁	2.58 ± 0.58 ^A	0.80 ± 0.65 ^{B**}
	d ₂	3.12 ± 0.40 ^A	0.50 ± 0.44 ^{B**}
OC (%)	d ₁	3.11 ± 0.18 ^{Ans}	3.66 ± 0.12 ^{Ans}
	d ₂	1.23 ± 0.05 ^B	2.72 ± 0.01 ^{A**}
K (Mg/kg)	d ₁	629.13 ± 25.08 ^B	671.73 ± 32.55 ^{A*}
	d ₂	557.67 ± 0.12 ^B	616.93 ± 26.45 ^{A**}
Soil texture	–	Sandy loamy	Sandy loamy

*d₁=0–20 cm, d₂=20–40 cm, **: Significant at 1% confidence, *: Significant at 1% confidence, ns: not significant. Similar letters had no significant difference at first and second soil layers ($p \leq 0.05$)

canopy cover (m^2/plot), density (number/plot), and basal area (cm^2/plot) of the shrubs were measured in plots.

2.3.2 Measuring nitrogen fixation (NF)

The soil samples were dried, and a part of each sample was transferred to the greenhouse for pot culturing to determine biomass production. The remaining part of the samples was bumped and passed through a 2-mm sieve.

Total soil N content was determined using the Kjeldahl method—wet oxidation [24]. The measurement of mineral N was taken using the method outlined by Keeney [25]. The amount of ON was calculated by subtracting MN from total N content.

2.4 The greenhouse experiments

The soil samples of the *H. halodendron* and *B. integerrima* species' rhizosphere and control area were transferred to the greenhouse. Plastic pots characterizing 21 cm in height and 15 cm in diameter were filled with soils. Fast-growing garden cress seeds (*Lepidium sativum* L.) were then cultured and grown in the pots. The experiment was conducted in a completely randomized design with four replications. Each pot contained 4 plants. Pots were irrigated regularly every 3 days. Greenhouse conditions (temperature = 25 °C; humidity = 70%) were controlled through experiments. After 30 days, *L. sativum* plants were harvested, dried in the shade, and weighed to determine plant biomass.

The total N content of *L. sativum* plants was obtained using a modified Kjeldahl method based on 0.5 g of dry plant material [26]. The N content was determined for plants in plastic pots.

2.5 Statistical analysis

Data were tested for the normality using the Kolmogorov–Smirnov test (KS test). Then, analysis of variance (ANOVA) was used to determine significant differences between treatment means, and post hoc tests were then performed using Duncan's grouping method. Also, a linear regression was performed between soil N and *L. sativum* biomass. Statistical analyses were performed through SPSS software (17.0 version).

3 Results

The growth rate of *B. integerrima* and *H. halodendron* was different in Mute and Ardeste areas during 2013–2019 (Fig. 1). However, characteristics such as plant density and canopy cover did not show significant changes during

the monitoring period. Height of shrubs showed the most changes during 2013–19 ($p < 0.05$).

The root zones of *H. halodendron* had significantly higher amounts of MN and ON compared to the root zones of *B. integerrima* and the soil between plant stands ($P \leq 0.01$). However, there were no significant differences in mineral N between the two species' root zones in both sites and root depths (Ranging 90–110 mg kg^{-1} ; $p \leq 0.05$). Comparisons of the amounts of ON and MN in the root zones of *H. halodendron* and *B. integerrima* and the soil between *H. halodendron* plants had fluctuations trend (Fig. 2 and Fig. 3).

Figure 3 shows the changes in MN under two plants' rhizosphere over time. Regardless of the species and soil depth, there was a minimum positive change in MN over time. The maximum and minimum changes were 0.25% year^{-1} and 0.93% year^{-1} in control patch of *H. halodendron* rhizosphere, respectively, both in 20–40 cm depth.

L. sativum biomass production cultivated in soil *H. halodendron*, *B. integerrima* rhizospheres and control indicated that there are significant differences in terms of soil N content that led to more plant growth and performance. The first evidence showed the topsoil had more positive effect on *L. sativum* biomass.

The soils under *H. halodendron* supported significantly higher *L. sativum* biomass than those under *B. integerrima* and between shrubs stands. Plant biomass was 177, 144, and 114 g for the first depth of the soil from *H. halodendron* and *B. integerrima* rhizosphere and the control, respectively, in Mute area in 2019. The corresponding values were 162, 126, and 87 g for the second depth of soil. *L. sativum* cultivated in soil *H. halodendron* rhizosphere showed the most biomass production (Table 2).

Liner correlation between N content of *L. sativum* and SON and MN of *H. halodendron* and *B. integerrima* rhizosphere is presented in Fig. 4. Based on the result, the higher N content in *L. sativum* was related to SON. In fact, this relation was significantly different based on N type ($p < 0.05$). However, higher levels of N and subsequently higher biomass production in tested plant were related to the soil of semi-arid region of Ardeste. Generally, higher relation was observed between plant N and ON of *H. halodendron* rhizosphere with a $R^2 = 0.606$ (Fig. 4).

4 Discussion

BNF is critical for ecosystem productivity [27]. In particular, it is important to know indigenous N fixing species of arid and semi-arid regions [18]. The results revealed that environmental variables create specific conditions for the NF and performance of plants. Based on the results,

Fig. 1 Changes in vegetative characteristics of *B. integrerrima* and *H. halodendron* during 2013–19 in Mute (a) and Ardeste (b). {HSh=Height of shrubs (cm/plot) CC=Canopy cover (m²/Plot), D=Density (Number/Plot), BA=Basal area (cm²/Plot)}

H. halodendron rhizosphere had more N content (SON, MN forms) than control areas. Moreover, *H. halodendron* rhizosphere had an ON content of 415–463% in topsoil and 460–512% time more than *B. integrerrima*. NF is a high energy-demanding process and microorganisms with this capacity have relatively high demands for ATP [7, 28], and therefore, indigenous legume species with

their adaptive mechanisms play an important role in NF in arid and semi-arid areas.

Both species affected soil N content. In fact, legume and non-legume plants influence soil N. However, a number of non-legume plants have evolved multiple strategies for NF [29], but their potential for N capturing and fixing is less than legume plants. Similarly, previous study reported

Fig. 2 Changes in SON in Mute (a) and Ardeste (b) through time. PR plant rhizosphere, C control

that different legume and non-legume plant species have a different NF potential in southern Japan [30]. On the one hand, this study revealed that MN and ON significantly changed in rhizosphere of species in two study areas under arid and semi-arid conditions. N in several forms are important considerations in studies of N processes in soil and related ecosystems, and its changes showed ecosystem dynamics under any environmental condition [31]. However, there was a significant difference in *H. halodendron* rhizosphere in terms of N content over time.

Conforming this, other researcher concluded that soil N content was affected principally by plant species and that successful long-term restoration improved the NF potential [7]. Another study in the western Great Basin, USA, also indicated that the non-fixing shrubs

consistently had lower mean N values than the same reference shrub species [32]. These species due to their capacity to fix N are essential components in natural and managed terrestrial ecosystems [13]. Therefore, *H. halodendron*, as a woody durable species with high NF potential, plays an important ecological role in soil N in these arid areas.

Moreover, soils collected from the root zones of *H. halodendron* and *B. integerrima* and control showed significant differences in terms of MN ($p < 0.02$). Previous study reported similar results in plant communities in areas with low plant productivity [33]. In previous study, it has been shown that N fixing *Robinia pseudoacacia* significantly improved the N in comparison with non-N fixing *Juglans regia* in semi-arid Loess Plateau region, China [34].

Fig. 3 Changes in soil mineral nitrogen in Mute (a) and Ard-este (b) through time. *PR* plant rhizosphere, *C* control

Investigation of vegetation characteristics of *H. halodendron* and *B. integerrima* showed significant differences in two sites during 2013–2019. It seems that the most changes were related to the environmental factors of precipitation and temperature in these arid and semi-arid areas. However, as vegetation characteristics were different, NF rates were also different through years. It seems that vegetation characteristics, i.e., biomass production and NF, are linked together. Similarly, it has been reported in other study that aboveground biomass, stand density, and basal area had a positive relation with NF in a nitrogen-fixing *Acacia auriculiformis* and non-nitrogen-fixing

Pterocarpus indicus [35]. In fact, due to their greater biomass that returns to the soil, large shrubs and trees have much greater effects on the soils than small shrubs and trees [36, 37]. The rates of NF on a 38-year-old *Alnus hirsuta* plantation in central Korea in which average nodule biomass was different based on growing season and average of 220 kg ha⁻¹ have been observed [38].

Regarding pot culturing results, soil had a significant effect on *L. sativum* biomass production and its N content. Soils of *H. halodendron*, *B. integerrima* rhizosphere and areas between plant stands (control) showed different and significant relation with plant N content ($p < 0.05$). These results

Table 2 *L. sativum* biomass production cultivated in soils of Mute and Ardeste

	Control		<i>H. halodendron</i>		<i>B. integerrima</i>		Sig
	0–20 cm	20–40 cm	0–20 cm	20–40 cm	0–20 cm	20–40 cm	
<i>Mute</i>							
2013	75.5 ± 11.0 ^{BCb}	55.0 ± 8.8 ^{Cc}	153.0 ± 21.0 ^{Ac}	114.5 ± 00.0 ^{Ad}	80.6 ± 17.6 ^{BCa}	65.8 ± 10.0 ^{Ca}	0.001**
2015	76.0 ± 12.3 ^{BCb}	54.2 ± 11.0 ^{Cc}	156.0 ± 24.5 ^{Ac}	132 ± 00.0 ^{Bc}	81.0 ± 15.5 ^{BCa}	66.4 ± 12.4 ^{Ca}	0.001**
2017	75.0 ± 10.8 ^{Bb}	56.1 ± 12.0 ^{Cc}	162.0 ± 26.8 ^{Ab}	139 ± 00.0 ^{Ab}	82.2 ± 19.2 ^{Ba}	71.0 ± 13.7 ^{BCa}	0.001**
2019	77.5 ± 15.0 ^{Bab}	58.0 ± 11.7 ^{Cc}	163.4 ± 31.0 ^{Ab}	145 ± 00.0 ^{Ab}	84.0 ± 21.0 ^{Ba}	71.4 ± 14.1 ^{BCa}	0.001**
<i>Ardeste</i>							
2013	81.2 ± 17.0 ^{Bab}	81.0 ± 17.4 ^{Bb}	158.7 ± 19.4 ^{Abc}	144 ± 00.0 ^{Ab}	85.0 ± 23.2 ^{Ba}	71.6 ± 15.2 ^{Ba}	0.002**
2015	82.0 ± 17.3 ^{Ba}	81.4 ± 17.5 ^{Bb}	165.5 ± 26.4 ^{Ab}	152 ± 00.0 ^{Aab}	85.3 ± 19.8 ^{Ba}	72.2 ± 14.8 ^{Ba}	0.002**
2017	84.0 ± 18.0 ^{Ca}	86.0 ± 18.3 ^{Cab}	178.3 ± 35.2 ^{Aab}	153 ± 00.0 ^{Bab}	86.4 ± 22.3 ^{Ca}	73.0 ± 18.0 ^{Ca}	0.001**
2019	86.0 ± 16.5 ^{CDa}	92.0 ± 17.6 ^{Ca}	189.4 ± 31.8 ^{Aa}	165 ± 00.0 ^{Ba}	89.5 ± 25.3 ^{CDa}	73.1 ± 17.0 ^{Da}	0.001**
Sig	0.024*	0.022*	0.002**	0.002**	0.562 ^{ns}	0.453 ^{ns}	–

*Uppercase and lowercase show the grouping in the row and column, respectively. **: Significant at 1% confidence, *: Significant at 1% confidence

support previous findings that due to higher NF potential, cultivated plant species had high biomass production [39, 40]. Soil N deficiency impaired plant N status and biomass yield [17, 41].

The relationship between soil N and plant production showed that soil N had a positive relation with *L. sativum* biomass production. However, the most positive relation was observed between soil ON and plant N content ($R^2 = 0.606$ and $R^2 = 0.585$ between plant N content and soil ON and MO, respectively). Similarly, in Fennoscandian region, the abundance of N sources in soil had a positive effect on performance and plant N content [33].

In arid and semi-arid ecosystems, however, soils have structural and microbial heterogeneity driven by sparse [42, 43] and restoration strategies that include native plants, including leguminous N fixing species, to rehabilitate degraded lands are efficient in reestablishing nutrient cycling processes [44]. On the other hand, N nutrition is a very complex that affects almost all physiological processes in plants for higher tolerance against environmental stress

[45, 46] which has double importance in arid and semi-arid lands under water deficiency.

H. halodendron has a positive effect on N accumulation in soil under arid and semi-arid conditions and therefore is one of the suitable species for plantation and rehabilitation of these areas. This species creates a natural and sparse forest in Mute and Ardeste areas. Conversion of natural forest may play significant role in the soil N cycle which completely affects ecosystem functions [47].

5 Conclusion

H. halodendron as an indigenous species had significant effects on soil N under arid and semi-arid conditions. *H. halodendron* had an effect of 400% on NF more than control *B. integerrima*. Maximum rates of 1.05% year⁻¹ and 1.54% year⁻¹ for SON and 0.50% year⁻¹ and 0.66% year⁻¹ for MN were observed in *H. halodendron* rhizospheres (0–40 cm) in arid and semi-arid areas, respectively. This specie also had a positive and significant effect on the biomass production and N content of *L. sativum* ($p < 0.02$; $R^2 = 0.606$ and 0.585 between SON and MN of shrubs rhizosphere, respectively, and *L. sativum* N content.

Fig. 4 Liner relation between N content of *L. sativum* and SON (a) and MN (b) of *H. halodendron* and *B. integerrima* rhizosphere

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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