

## Response to referees' comments (SE-2014-81)

Manuscript title: Changes in soil organic carbon and nitrogen capacities of *Salix cheilophila* Schneid. along a revegetation chronosequence in semi-arid degraded sandy land of the Gonghe Basin, Tibet Plateau

Authors: Y. Yu and Q.Z. Jia

Dear Editor,

Thank you very much for your thoughtful comments and considerate suggestions for our manuscript. These comments are valuable and helpful for improving our manuscript. We have made careful modifications and revisions on the original manuscript in response to all the reviewers' comments and editor's suggestions. We hope the new version of the revised manuscript would meet the Journal's standard. Answers to referee's questions are bold.

### **Anonymous referee #1:**

General comments: The paper looks fine for me I just suggested to use very recent citations to make the paper more scientifically sound And also some improvements in the figures The paper is a good contribution.

In the introduction you need some citations to support your initial sentence/ideas and you need some citations to show this to the reader

Figure 5 and 6 I suggest to reduce the decimals to two in the legend and axis

Figures 2, 3 and 4 A color figure will be of great help for the reader

Specific comments:

1. In the introduction you need some citations to support your initial sentence/ideas and you need some citations to show this to the reader.

Response: **Thanks very much for the reviewer's comment. We have carefully revised the introduction of the manuscript, and improved the quality as well as add the recent citations according to the comments. (See page 2, line 17-18, page 3, line 4-5)**

2. Figure 5 and 6 I suggest to reduce the decimals to two in the legend and axis.

Response: **We have revised figure 5 and 6. The decimals in the legend and axis were unified to two places. (See supplement figure 5 and 6).**

3. Figures 2, 3 and 4 A color figure will be of great help for the reader.

Response: **Thanks for the reviewer's considerate advice. We added colors to the figures. (See supplement figure 2, 3 and 4).**

### **Anonymous referee #2:**

Generally comments: This manuscript described the SOC and TN change of restoration in a sandified and desertified region in Qinghai-Tibet plateau which is very important for ecological restoration in this area. Such study is very few in this region. Generally, the manuscript is well structured and organized. Also, the results were expressed very well. I recommend this manuscript could be accepted with minor modification.

Specific comments:

1. afforestation with *Salix cheilophila* should be restoration with *Salix cheilophila*.

Response: **Thanks very much for the reviewer's precious comment. The "afforestation with *Salix cheilophila*" was all changed to "restoration with *Salix cheilophila*" in the revised manuscript.**

2. I suggest the discussion and results could be separated into two parts.

Response: **Thanks very much for the reviewer's considerate suggestion for separating the results and discussion parts. In most of the paper, these two parts were separated. In this manuscript, we suppose that it is more appropriate to combine the two parts for considering the integrity of the analysis of SOC and TN changes along a revegetation chronosequence. Based on the result of BD and biomass, we discussed the relationship between BD/biomass and SOC respectively, which might improve the SOC analysis in the following section. We hope that we are following your point.**

3. In Figure 1, the sites could be further located in detail.

Response: **Thanks very much for the reviewer's comment. The study site was revised and we added the specific information. (See supplement figure 1).**

## **Editor's comments:**

1. Objectives should be separated in a new paragraph at the end of the introduction section.

Response: **Thanks very much for the Editor's thoughtful comment. The objectives were separated in a new paragraph. (See page 4, line 25)**

2. Page 2377, lines 20-22: The three 20 m x 20 m and five 1 m x 1 m plots were selected randomly, but how? Using randomly generated coordinates or any other process?

Response: **Thanks very much for the Editor's comment. After a careful examination of the restoration history including method for afforestation, similar topography, soil texture and site condition, we identified the sampling field for four representative restoration periods. Three 20 x 20 m plots of each restoration periods were immediately adjacent together, the 11-year stand is approximately 0.2 km southwest of the 6-year, the 16-year stand is approximately 1.5 km southeast of 6-year, while the 21-year stand is about 0.8 km in the southeast of 16 year. The general situation of the experiment site please see supplement table 1. Meanwhile, we identified five 1 m x 1 m plots with in each fields and sampled for both accumulated litter and understory plant biomass, the plots were at least 5 m apart from each other and 5 m away from boundary. To some degree, this is the base principle of our randomly sampling. Finally, thanks for your valuable comments once again. We changed the statement in the revised manuscript. (See page5, line 27 and 28, page 6, line 1-7)**

3. Page 2378, lines 1-2: I suggest re-writing depth classes: 0-10, 10-20, 20-30, 30-50, 50-100, 100 -150 and 150-200 cm. Much more easy to understand.

Response: **Thanks very much for the Editor's thoughtful comment. We re-wrote the depth classes in the revised manuscript. (See page7, line 20-21)**

4. Page 2385, line 19. Please, add some recent references for SOC studies in depth layers

Response: **Thanks very much for the suggestion. We added the citation in the revised manuscript. (See page 12, line 27 and page18, line 7-18)**

1 **Changes in soil organic carbon and nitrogen capacities of**  
2 ***Salix cheilophila* Schneid. along a revegetation**  
3 **chronosequence in semi-arid degraded sandy land of the**  
4 **Gonghe Basin, Tibet Plateau**

5  
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13  
14 **Abstract**

15 The Gonghe Basin is a sandified and desertified region of China, but the distribution of soil  
16 organic carbon (SOC) and total nitrogen (TN) along the cultivation chronosequence across  
17 this ecologically fragile region is not well understood. This study was carried out to  
18 understand the effects of restoration with *Salix cheilophila* for different periods of time (6, 11,  
19 16, 21 years) to test whether it enhanced C and N storage. Soil samples, in four replications  
20 from seven depth increments (0–10, 10–20, 20–30, 30–50, 50–100, 100–150 and 150–200  
21 cm), were collected in each stand. Soil bulk density, SOC, TN, aboveground biomass and root  
22 biomass were measured. Results indicated that changes occurred in both the upper and deeper  
23 soil layers with an increase in revegetation time. The 0–200 cm soil showed that the 6-year  
24 stand gained 3.89 Mg C ha<sup>-1</sup> and 1.00 Mg N ha<sup>-1</sup>, which accounted for 40.82% of the original  
25 SOC and 11.06% of the TN of the 0-year stand. The 11-year stand gained 7.82 Mg C ha<sup>-1</sup> and

1 1.98 Mg N ha<sup>-1</sup> in the 0–200 cm soil layers, accounting for 58.06% of the SOC and 19.80% of  
2 the TN of the 0-year stand. The 16-year stand gained 11.32 Mg C ha<sup>-1</sup> and 3.30 Mg N ha<sup>-1</sup> in  
3 the 0–200 cm soil layers, accounting for 66.71% of the SOC and 21.98% of the TN of the  
4 0-year stand. The 21-year stand gained 13.05 Mg C ha<sup>-1</sup> and 5.45 Mg N ha<sup>-1</sup> from the same  
5 soil depth, accounting for 69.79% of the SOC and 40.47% of the TN compared with the  
6 0-year stand. The extent of these changes depended on soil depth and plantation age. The  
7 results demonstrated that as stand age increased, the storage of SOC and TN increased. These  
8 results further indicated that restoration with *S. cheilophila* has positive impacts on the  
9 Gonghe Basin and has increased the capacity of SOC sequestration and N storage. Shrub's  
10 role as carbon sink is compatible with system's management and persistence. The findings are  
11 significant for assessing C and N sequestration accurately in semi-arid degraded high-cold  
12 sandy regions in the future.

## 13 **1 Introduction**

14 Arid and semi-arid regions cover ~30% of the terrestrial land around the globe and  
15 desertification affects over 250 million people (Lal, 2001; Reynolds et al., 2007; Lal, 2009;  
16 Allington and Valone, 2010). In the largest developing country, China, the most typical and  
17 serious form of land degradation is desertification (König et al., 2012; Wang et al., 2013;  
18 Zhao et al., 2013). China is the country with the largest area of desertified or sandified lands  
19 in the world. According to statistics, China has a total desertified land area of  $26.237 \times 10^5$  km<sup>2</sup>  
20 covering 27.33% of the national territory and a total sandified land area of  $17.311 \times 10^5$  km<sup>2</sup>  
21 covering 18.03% of the national territory and which are under threat of land degradation by  
22 the end of 2009 (State Forestry Administration, 2011). Desertification is the degradation of  
23 land in arid, semi-arid and sub-humid dry areas resulting from various factors, including  
24 climatic variations and human activities (UNEP, 1994). It results in soil degradation and  
25 severe decreases in land potential productivity. With the exception of land degradation,  
26 desertification promotes atmospheric emission of soil C and N as greenhouse gas (Breuer et  
27 al., 2006). Measures such as artificial reforestation and grass plantation have worked to  
28 improve the ecological benefits of sandstorm control to reduce the damage from sandstorms.  
29 Revegetation of degraded land is a major global issue, which has been shown to improve and

1 restore some of the ecosystem services both of the physical and biological processes. It has  
2 been widely recognized that revegetation is an effective measure for soil and water  
3 conservation, increasing C and N storages and improving land productivity (Grünzweig et al.,  
4 2003; Cao et al., 2008; Hu et al., 2008; Lal, 2009; Cao et al., 2011; Li et al., 2012; Barua and  
5 Haque, 2013; Su et al., 2013; Jaiarree et al., 2014; Guzman et al., 2014; Srinivasarao et al.,  
6 2014). In desertified areas of northwest China, establishing artificial vegetation and bans on  
7 grazing are commonly adopted measures for combating desertification and restoring  
8 vegetation. It not only resists the spread of desertification but also restores ecosystem  
9 processes that could potentially yield significant gains in nutrients storage (Zhao et al., 2007;  
10 Huang et al., 2012). Therefore, land use and management practices to sequester soil organic  
11 carbon (SOC), including afforestation and revegetation, are the driving forces that could  
12 determine the transition of desertification regions from a C source to a C sink or vice versa.  
13 For this reason, the effects of revegetation on soil C and N contents in degraded land have  
14 become a concern in recent years.

15 Revegetation on a large-scale in degraded arid and semi-arid lands is likely to have far  
16 reaching consequences on the global C cycle and climate change (Lal, 2009). To know the  
17 changes in soil C and N content is not only critical to determining the soil physiochemical  
18 properties but also to quantifying the influence of changing rates of C and N cycling and  
19 storage (Liu et al., 2002). It has been reported that chronosequence or successional stage may  
20 be a critical factor affecting changes in C stock and allocation among the different ecosystem  
21 components (Li et al., 1997; Zhang et al., 2005; He et al., 2012). Wang (2009) observed that a  
22 significant difference in SOC occurred in a semi-arid grassland of an undisturbed steppe, a  
23 28-year crop land and a 42-year crop land and the changes depended on soil depth and land  
24 age. Chen (2010) and Li (2012) reported that SOC and N increased significantly in different  
25 depths with plantation age of Mongolian pine in semi-arid degraded sandy land. Zhou (2011)  
26 investigated the dynamics of soil C and N accumulation over 26 years under controlled  
27 grazing in a desert shrubland. Su (2005) found that after planting the shrubs *Caragana*  
28 *microphylla* Lam. and *Artemisia halodendron* Turcz. ex Bess on shifting sand dunes, SOC  
29 and N significantly increased in two upper soil layers (0–5 cm and 5–20 cm) in semi-arid

1 Horqin sandy land. Information on SOC and N concentration in a long-term revegetation  
2 chronosequence is necessary to identify the strategies of degraded land recovery. Despite an  
3 increasing number of related studies, the effect of *Salix cheilophila* on soil improvement still  
4 remains poorly understood.

5 The Gonghe Basin, located in the northeast Tibet Plateau (35°27' to 36°56' N, 98°46' to  
6 101°22' E), is one of the most seriously desertified and ecologically fragile high-cold regions  
7 in the Qing Hai province of China. Arbitrary land use and several decades of overgrazing  
8 have led to land degradation and desertification. Frequent sandstorms happened and  
9 desertification occurred during the last century. Semi-arid steppe, sandland and shrubland are  
10 widely distributed in the Gonghe Basin. Based on the geographic-ecological similarity, one  
11 effective approach to improve the fragile ecological environment and control for  
12 desertification is to select shrub species that have excellent adaptability and characteristics  
13 under natural ecological conditions. Large areas of trees and shrubs have been planted in this  
14 region since the 1980s. *Salix cheilophila* is one of the shrub species growing well in degraded  
15 land and it can be used for multiple shelterbelts and desertification control. *S. cheilophila*  
16 Schneid. is a member of the Salix Family (Salicaceae), is a Chinese endemic species which  
17 adapts well to windy and sandy environments and is widely distributed throughout the  
18 Northwest of China, especially in the Qing Hai province and Tibet. Because of its adaptability  
19 in harsh environmental conditions, *S. cheilophila* is widely cultivated in revegetation  
20 programs to control desertification in the Gonghe Basin. The metabolic activities of *S.*  
21 *cheilophila* have been extensively studied by H. Liu (2012) and L. Liu (2012); however, there  
22 remains little knowledge about *S. cheilophila* enhancing soil SOC and N along a  
23 chronosequence in this region. It was hypothesized that SOC and N allocation changes with  
24 increasing stand age of *S. cheilophila* and soil fertility significantly increases over time.

25 The objectives of our study were to investigate the soil physicochemical properties and  
26 quantify the effects of vegetation restoration on the SOC and N in *S. cheilophila* plantations  
27 and in lowland among sandy dunes of the Gonghe Basin. Results from this study can provide  
28 base data for the parameterization of regional models that can be used to determine SOC and  
29 N storages under *S. cheilophila* plantations and provide the basis for soil improvement of

1 high-cold sandy land ecosystem services.

## 2 **2 Materials and methods**

### 3 **2.1 Study area**

4 The study was conducted at the Gonghe Desert Ecosystem Research Station (latitude N  
5 36°19', longitude E 100°16' and altitude 2871 m), which was constructed by the Chinese  
6 Academy of Forestry and the Desertification Combating Station of Qinghai Province (Fig. 1).  
7 It is one of the stations in the Chinese Desert Ecosystem Research Network located in the  
8 Gonghe Basin on the northeast part of the Tibetan plateau. The area has a strong continental  
9 semi-arid climate. The growing season is from June to September. The mean annual  
10 precipitation is ~246.3 mm, more than 75% of which falls during the growing season, and the  
11 mean annual air temperature is 2.4 °C. The mean annual potential transpiration is 1716.1 mm,  
12 the mean annual number of windy days is 50.6 d and the primary wind direction is  
13 north-northwest. The mean annual wind speed is 2.7 ms<sup>-1</sup> and the mean length of the  
14 frost-free season is 91 d. The vegetation in the desertified sandy land is generally dominated  
15 by psammophytes including grasses (e.g., *Leymus secalinus*, *Orinus kokonorica*, *Stipa*  
16 *capillata* and *Thermopsis lanceolata*) and shrubs (e.g., *Caragana intermedia*, *Salix*  
17 *cheilophila* and *Tamarix chinensis*). *C. intermedia*, a leguminous shrub, is the dominant shrub  
18 species on semifixed and fixed sandy dunes. *Salix cheilophila* is the dominant shrub species  
19 on land between dunes. Both of them adapt well to the sandy environment, and have been  
20 widely used in vegetation re-establishment programs, such as artificial shelter belts, since the  
21 1980s. Four stands of *S. cheilophila* of different ages (6, 11, 16, and 21 years) were identified.  
22 A plot (0 years old) between dunes was used as a control. All of the stands located in the land  
23 between dunes had only rarely been disturbed by human activities and had naturally  
24 regenerated after revegetation. The main type of soil in the research region is sandy loam, and  
25 clay exists at different soil depth.

### 26 **2.2 Soil sampling and laboratory analysis**

27 The field measurements and sampling were completed in the growing season of 2011 and



1 2012 (June to August). Three 20-m × 20-m plots of each restoration periods were immediately  
2 adjacent together, the 11-year stand is approximately 0.2 km southwest of the 6-year, the  
3 16-year stand is approximately 1.5 km southeast of the 6-year, while the 21-year stand is  
4 about 0.8 km in the southeast of the 16 year. In each of the plantation plots, tree basal  
5 diameter and average tree height for all of the live *S. cheilophila* were recorded using a  
6 diameter tape, and canopy height was estimated using a clinometer for all trees within each  
7 plot. Meanwhile, we identified five 1-m × 1-m plots with in each fields and sampled for both  
8 accumulated litter and understory plant biomass, the plots were at least 5 m apart from each  
9 other and 5 m away from boundary. During the study, four trees representing the respective  
10 stand-specific basal diameter and height range were selected. A depth of 0–200 cm was  
11 divided into seven layers (0–10, 10–20, 20–30, 30–50, 50–100, 100–150 and 150–200 cm),  
12 and samples were taken with a 6-cm diameter soil core on the edge of the south crown of each  
13 standard tree. Therefore, in every plot, a total of 28 composite soil samples were obtained for  
14 each soil layer with a total of 112 samples across all plots. The samples were sealed in plastic  
15 bags and transported to the laboratory. Soil bulk density (BD) of each depth increment for  
16 every sampling site was measured using the core method (stainless steel cylinders with a  
17 volume of 100 cm<sup>3</sup>). All soil samples were air dried and visible plant material was removed,  
18 then they were sieved to 0.5 mm for SOC and TN measurements. In each plot, roots of four  
19 samples were excavated manually from each of the soil layers. All root samples were  
20 transported to the laboratory, and carefully washed on a 60 mm sieve to separate the roots  
21 from the soil at once. All washed roots were weighed after oven drying at 65 °C for 48 hours.  
22 Total SOC was determined by loss on ignition at 500 °C (Storer, 1984). Total N concentration  
23 was measured by the Kjeldahl procedure (Bremner et al., 1996).

### 24 **2.3 Calculations and data analysis**

25 The SOC and TN at each depth were obtained by the sum of organic C and N stocks of the  
26 seven depths. The SOC mass per unit surface area (kg m<sup>-2</sup>) of a profile is calculated as the  
27 weighted average of the SOC mass density of every depth, where the thickness of the horizon  
28 is the weighting factor, multiplied by the reference depth (Meersmans et al., 2008; Han et al.,  
29 2010). For each depth interval, SOC and TN stocks were calculated with the following

1 equation:

$$2 \quad S = EC \times BD \times T \times k \times 10^{-6},$$

3 where  $S$  is the element stocks ( $\text{kg m}^{-2}$ ),  $EC$  is the element concentration ( $\text{g kg}^{-1}$ ),  $BD$  is the  
4 bulk density ( $\text{g cm}^{-3}$ ),  $T$  is the thickness of the horizon and  $k$  is the area multiplier.

5 This study did not involve replicated stands of the same age with a similar stand composition,  
6 soil type and environmental conditions, because of the complexity of the study site in this area.  
7 Data were analyzed to provide mean and standard error for each variable measured at every  
8 depth in each stand. Analysis of variance was performed using the MIXED procedure in SAS  
9 that computes Wald-type F-statistics using generalized least squares (GLSE) based on  
10 restricted maximum likelihood estimates of the variance components (Littell et al., 1996). In  
11 the case of significant differences in the Wald-F-statistic at  $P < 0.05$ , treatment means were  
12 compared using a two-sided t test. The regression model was determined with Matlab 8.0  
13 software. All statistical analyses were conducted with the SAS software package (SAS,  
14 Institute Inc. 2000).

### 15 **3 Results and discussion**

#### 16 **3.1 Soil bulk density**

17 Soil BD plays a critical role in the assessment of SOC contents. Table 1 shows that the BD  
18 values are significantly different in different stand ages and marked differences were found  
19 among the different soil depths. This indicated that the soil BD of the 21-year stand was lower  
20 compared with other stand ages in each of the seven depths (i.e. 1.49, 1.39, 1.47, 1.46, 1.47,  
21 1.52 and 1.53  $\text{g cm}^{-3}$  in the 0–10, 10–20, 20–30, 30–50, 50–100, 100–150 and 150–200 cm  
22 depths, respectively). The mean BDs decreased with the extension of restoration time. At  
23 0–10 cm, the 16- and 21-year stands were significantly different to the other stand ages but  
24 not from each other. The 11-year stand was significantly lower than the 6 and 0 year stands  
25 but there was no significant difference between the 0- and 6-year stands. At 10–20 cm, the  
26 21-year stand was significantly lower than any other stand and the 16-year stand showed no  
27 significant difference compared with the 11-year stand but was significantly lower than the 6-  
28 and 0-year stands, which in turn were significantly different from each other. At 20–30 cm,

1 the 21-year stand was significantly lower than the other stand ages and there was no  
2 significant difference between the 6-, 11- and 16-year stands. The 11-year stand showed no  
3 difference with the 0-year area but the 6- and 16-year areas were significantly lower than the  
4 0-year area. At 30–50 cm, the only difference from 20–30cm was that there was no significant  
5 difference among the 6-, 11-, 16- and 21-year areas; however, all of them were significantly  
6 different from the 0-year area, which showed the same changes at 50–100 cm.

7 In subsoil, significant differences in soil BD were also exhibited among the different stand  
8 ages. The 21-year stands showed no significant differences from the 16-year stands but were  
9 significantly lower than the other stand ages. There was no significant difference among the  
10 0-, 6- and 11-year stands at 100–150 cm. At 150–200 cm, significant differences existed  
11 among the stand ages. The 21-year stand was significantly different to all stand ages except  
12 16 years and there was no significant difference among the 0-, 6-, 11- and 16-year stands. The  
13 results indicated that vegetation restoration could affect the soil BD, possibly because of the  
14 plant roots (Ryan & Law, 2005).

15 It is also widely believed that soil BD declines with an increase in soil organic matter because  
16 of the increase in porosity volume (Whalen et al., 2003). Therefore, the linear relationship  
17 between soil BD and SOC was established in various ecosystems. Prior to this study, no data  
18 existed on the relationship between BD and SOC for soils in High-Cold Sand land of the  
19 Gonghe Basin. The relationship in the *S. cheilophila* chronosequence was modeled with  
20 SigmaPlot 2011, and it was found that there was a linear relationship that can be described by  
21 the following equation (Fig. 2):

$$22 \text{ SOC} = 39.129 - 22.187 \text{ BD} \quad (R^2 = 0.247, P < 0.001).$$

### 23 **3.2 Root biomass and aboveground biomass**

24 The data in Table 2 clearly show that revegetation led to significant differences in both  
25 aboveground and root biomass, and that root biomass in the deep soil layers also increased  
26 significantly with the extension of restoration time. The root biomass in differently-aged  
27 stands changed significantly with an increase in depth. The aboveground biomass increased  
28 along the chronosequence, and was 776.40 g m<sup>-2</sup> for the 6-year, 1011g m<sup>-2</sup> for the 11-year,

1 2098g m<sup>-2</sup> for the 16-year and 2963g m<sup>-2</sup> for the 21-year stands. Additionally, the root  
2 biomass also showed an increasing trend: 281.64 g m<sup>-2</sup> for the 6-year, 363.04g m<sup>-2</sup> for the  
3 11-year, 811.54g m<sup>-2</sup> for the 16-year, and 1120.61g m<sup>-2</sup> for the 21-year stands; this was  
4 significantly different at different soil depths. The aboveground biomass was nearly three  
5 times as large as the root biomass. Therefore, both the aboveground and the root biomass  
6 were the dominant source for soil C input in semi-arid degraded sandy land of the Gonghe  
7 Basin.

8 The significant increase in total C input with restoration time in the semi-arid degraded sandy  
9 area indicated that afforestation is an effective option to sequester C, which could further  
10 increase C influx through more efficient plant use of resources for primary production  
11 (Nosetto et al., 2006; Li et al., 2012). Therefore, the increase in SOC and N input will  
12 subsequently result in increased SOC and N storage.

### 13 **3.3 Soil organic C and N concentration**

14 The SOC and N storage increased significantly with plantation age but there were different  
15 changes as soil depth increased (Fig. 3). The mean was highest but most variable in the  
16 topsoil layer and dropped significantly in the subsoil layer (>100 cm). For the total study area,  
17 the SOC concentrations peaked at 0–10 cm except at 6 and 0 years, which have the highest  
18 amount of SOC at 10–20 cm. For the TN concentration, the 16- and 21-year stands peaked in  
19 the surface soil and 0-, 6- and 11-year stands have the highest amount at 10–20 cm. The SOC  
20 and TN concentrations were markedly altered by the extension of restoration.

21 In the top 10 cm, SOC was significantly greater in the 21-year stand than in the other stands  
22 and the SOC increased significantly with the extension of restoration time. At 10–20 cm,  
23 there were no significant differences between 16- and 21-year stands, but the SOC content  
24 was significantly greater in both of these than in the other aged stands. Although the SOC  
25 content in the 6-year stand was also significantly greater than in the 0-year stand, there was no  
26 significant difference between the 6- and 11-year stands. At 20–30 cm, the SOC content of  
27 the 21-year stand was significantly greater than that of any other and the 11-year stand  
28 showed no significant difference from the 0-year stand, but was significantly lower than the

1 6-year stand. At 30–50 cm, the SOC content in the 21-year stand was not significantly  
2 different from the 16-year stand but was significantly greater than for the other ages. There  
3 was no significant difference among the SOC contents of the 6-, 11- and 16-year stands,  
4 which were significantly greater than the 0-year stand. At 50–100 cm, the SOC content of the  
5 21-year stand was significantly greater than that of the 11-year stand, but was not  
6 significantly different from that of the 16-year stand. There was also no significant difference  
7 between the SOC contents of the 16- and 11-year stands, which were significantly higher than  
8 those of the 6- and 0-year stands.

9 The SOC storage in the deep soil showed a significant difference. The SOC in the 21-year  
10 stand showed no significant difference from the 16-year stand, and both of them were  
11 significantly higher than those in the 6- and 0-year stands, which showed the same changes at  
12 150–200 cm. There was no significant difference among the SOC contents of the 11-, 6- and  
13 0-year stands at 100–150 cm and that of the 11-year stand was significantly different to the  
14 other stand ages at 150–200 cm.

15 The patterns for TN concentration were not substantially different from those for SOC (Fig.  
16 4). In particular, although the 0–10 cm layer showed the same variation trend as SOC, there  
17 were significant differences in each stand. The TN in the 21-year stand was significantly  
18 greater than in the 16-year stand and there was no significant difference among the 11-, 6- and  
19 0-year stands, which were significantly lower than the 16-year stand. At 10–20 cm, there was  
20 no significant difference in TN among the 16-, 11- and 6-year stands, which were  
21 significantly greater than that in the 0-year stand and lower than that in the 21-year stands.  
22 The 20–30-cm and 30–50-cm layers showed the same changes in TN as the surface layer. At  
23 50–100 cm, the TN content of the 21-year stand was significantly greater than those of the  
24 other stands, which were not significantly different from each other. At 100–150 cm, there  
25 was a significant difference between the TN of the 11-year and other stands and the 16- and  
26 21-year stands were significantly greater than the 6- and 0-year stands. At 150–200 cm, there  
27 was no significant difference in TN content among the 11-, 16- and 21-year stands, which  
28 were significantly greater than those of the 6- and 0-year stands.

29 The higher SOC and TN content in the upper soil layer than the subsoil layer could be

1 explained by the root growth and decay process. It is widely accepted that plant roots play an  
2 important role among the various factors influencing soil structural porosity, especially the  
3 fine roots. Most of the roots were located in the upper soil. With the extension of restoration  
4 times, the vertical distribution and biomass of the roots increased, soil N was usually moved  
5 by roots from subsoil layers to the surface during plant growth, and the soil C and N were  
6 retained when the roots died, which resulted in increased C and N concentrations. It was  
7 found that the vertical distribution at 21 years could reach 200 cm. Moreover, the growth of  
8 the root system led to the changes of BD, which could promote the soil organic matter storage  
9 and total nitrogen content. Therefore, models simulated the changes of SOC and BD with the  
10 extension of stand age and depth were established (Fig. 5), using the SOC as the dependent  
11 variable (z), the BD and stand age as independent variable (x) and (y) respectively, the  
12 regression model was established as follow:

$$13 \quad z = -180.253 + 1.2x + 255.136y - 0.011x^2 - 0.474xy - 89.186y^2 \quad (R^2 = 0.458, P < 0.01)$$

14 when used the BD (x) and depth (y) as independent variable, the model was described as:

$$15 \quad z = -359.406 - 0.193x + 518.887y + 0.003x^2 + 0.078xy - 182.25y^2 \quad (R^2 = 0.521, P < 0.01)$$

16 The model of SOC and TN with the extension of stand age and soil depth also established  
17 (Fig. 6), using the SOC as the dependent variable (z), the TN and stand age as independent  
18 variable (x) and (y) respectively, the regression model was established as follow:

$$19 \quad z = -2.611 + 75.486x + 0.613y + 1867.623x^2 - 6.634xy - 0.011y^2 \quad (R^2 = 0.392, P < 0.01)$$

20 when used the TN (x) and depth (y) as independent variable, the model was described as:

$$21 \quad z = -3.668 + 368.861x - 0.009y - 2186.34x^2 - 0.965xy + 0.001y^2 \quad (R^2 = 0.427, P < 0.01)$$

22 The results indicated that afforestation could affect the BD and especially the shrub could  
23 reduce it evidently, the difference in the BD can be caused by the root. Moreover, the content  
24 of SOC and TN increased with the BD decreased.

### 25 **3.4 Soil organic C and N stocks or losses and gains of *Salix***

26 Table 3 shows the gains and losses of the SOC and TN in different stands relative to the  
27 0-year stand, based on calculations in which the BD variability, SOC, TN contents and depth  
28 were taken into account. The results indicated that the 6-year stand gained  $3.89 \text{ Mg C ha}^{-1}$  and

1 1.00 Mg N ha<sup>-1</sup> in the 0–200-cm soil layers, which accounted for 40.82% of the original SOC  
2 and 11.06% of the TN of the 0-year stand. The 11-year stand gained 7.82 Mg C ha<sup>-1</sup> and 1.98  
3 Mg N ha<sup>-1</sup> in the 0–200 cm soil layers, accounting for 58.06% of the SOC and 19.80% of the  
4 TN of the 0-year stand. The 16-year stand gained 11.32 Mg C ha<sup>-1</sup> and 3.30 Mg N ha<sup>-1</sup> in the  
5 0–200 cm soil layers, accounting for 66.71% of the SOC and 21.98% of the TN of the 0-year  
6 stand. The 21-year stand gained 13.05 Mg C ha<sup>-1</sup> and 5.45 Mg N ha<sup>-1</sup> from the same soil depth,  
7 accounting for 69.79% of the SOC and 40.47% of the TN compared with the 0-year stand.

8 Although the SOC and TN increased with stand age, different stages showed differences with  
9 soil depth. These results indicated that the 11-year stand lost 0.02 Mg C ha<sup>-1</sup> at 20–30 cm,  
10 decreased 4.5% compared to the 0-year stand. The soil has strong heterogeneity in arid and  
11 semi-arid regions. The root, which could be considered as the “bio-management” in the harsh  
12 environment, was the primary cause lead to the contents accumulation and consumption of the  
13 SOC and TN in different depth and stand age. Laclau (2003) and Li (2012) found that because  
14 of the biomass accumulations, soil organic matter increases with the extension of the  
15 revegetation time, in semi-arid areas. The present results are consistent with the findings of Su  
16 and Zhao (2003), who reported higher SOC in stands of *C. microphylla* shrub than in active  
17 sand dunes. Wei (2010) compared the distribution of SOC and N in soils under canopies and  
18 in outer tree canopies in semi-arid areas and found that dry climate, low C soils had a  
19 potential for C sequestration after grassland to woodland conversion. Hu (2008) documented  
20 a significant potential for soil C sequestration with afforestation in Horqin Sandy Land and Li  
21 (2012) revealed that Mongolian pine plantations in Horqin Sandy Land have a great potential  
22 to sequester C, which agreed with the present research. The Gonghe Basin has experienced  
23 intensive desertification in recent decades. *S. cheilophila* also has a great potential to  
24 sequester C. Therefore, it is important to comprehensively evaluate the effects of these  
25 plantations on ecosystem C sequestration in the Gonghe Basin. Although depth research on  
26 soil C studies varies (Guo and Gifford, 2002; Post and Kwon, 2008; Fu et al., 2010;  
27 Muñoz-Rojas et al., 2012a; 2012b; 2013; Parras-Alcántara et al., 2013), many studies have  
28 only considered SOC changes in the upper soil layers to investigate the impacts of land use  
29 change on soil properties and C storage. The subsoil also has a large SOC storage capacity

1 (Jobbágy and Jackson, 2000; Knops and Bradley, 2009; Carter and Gregorich, 2010; Chang et  
2 al., 2012). Therefore, more studies focusing on the subsoil SOC are necessary to accurately  
3 evaluate the changes in soil C pools following afforestation. The present results showed that  
4 significant responses occurred in the subsoil layer because of root distributions. In light of  
5 global warming, scientists have recognized the potential of soil as a C sink to counteract the  
6 increasing trend of atmospheric CO<sub>2</sub> concentration (Grace, 2004). Therefore, revegetation of  
7 degraded land, especially in desertified or sandified lands such as those in the Gonghe Basin,  
8 is an effective way not only to combat desertification but also to provide a C sink.  
9 Understanding the impact of revegetation and afforestation on the SOC storage and increasing  
10 the capability of soil C sequestration is a challenge for the future.

#### 11 **4 Conclusions**

12 This study demonstrated the significant increases in SOC and TN over time in *S. cheilophila*  
13 plantation soils in the Gonghe Basin of Qinghai, China. The establishment of *S. cheilophila* in  
14 the semi-arid high cold sandy land had positive impacts on the soil C sequestration and N  
15 storage. Soil organic C and TN increased significantly with plantation age. The difference  
16 indicated that the inputs of aboveground and root biomass were sufficient to increase the SOC  
17 and TN with the extension of revegetation time. The responses were observed among  
18 different stand ages not only in the top soil layer but also in the deeper soil. Plant roots played  
19 an important role in soil C sequestration especially in the study area characterized by the low  
20 SOC because of the sandy soil texture. It is necessary to focus on the changes in SOC in the  
21 deeper soil layers to assess C sequestration accurately. This study identified that restoration  
22 with *S. cheilophila* in high-cold sandy land of the Gonghe Basin is a positive way to improve  
23 soil quality and prevent desertification in these semi-arid regions.

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1 **Acknowledgements**

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5 for their constructive comments and suggestions.

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1 Table 1. Soil bulk density ( $\text{g cm}^3$ ) in different stand ages at different soil depth.

Depth /cm	Stand age /yr				
	0	6	11	16	21
0-10	1.56±0.01Aab	1.54±0.03ABc	1.53±0.01Bbc	1.51±0.01Cb	1.49±0.02Cbc
10-20	1.54±0.02Aa	1.44±0.04Ba	1.42±0.02BCa	1.41±0.01Ca	1.39±0.01Da
20-30	1.58±0.01Ac	1.53±0.01Bc	1.57±0.01ABc	1.53±0.03Bb	1.47±0.02Cb
30-50	1.56±0.02Abc	1.48±0.05Bab	1.51±0.02ABb	1.47±0.06Bab	1.46±0.01Bb
50-100	1.57±0.01Abc	1.52±0.02ABbc	1.50±0.05ABb	1.49±0.04Bab	1.47±0.02Bb
100-150	1.57±0.02Abc	1.55±0.02ABc	1.56±0.01ABc	1.53±0.04BCb	1.52±0.01Cd
150-200	1.57±0.02Abc	1.57±0.02Ac	1.57±0.01Ac	1.56±0.02ABb	1.53±0.03Bd

2 Different uppercase letters indicate significant differences in different stand ages, different  
 3 lowercase letters indicate significant differences in different soil depths ( $P < 0.05$ ).

4



1 Table 2. Aboveground and root biomass in different stand ages of *S. cheilophila*.

Age	Above-ground residue g m <sup>-2</sup>	Root biomass/ g m <sup>-2</sup>							
		0-10	10-20	20-30	30-50	50-100	100-150	150-200	total
6	776.40±21.14a	55.03±0.51a	60.12±4.14a	54.88±4.73a	45.46±2.69a	36.19±3.23a	30.22±2.59a	—	281.64±7.10a
11	1011.73±18.92b	69.16±3.21b	77.59±2.39a	71.66±2.02b	60.35±2.57b	48.33±2.93b	36.32±2.63a	—	363.04±4.81b
16	2098.19±75.72c	135.50±5.60c	149.19±5.00b	154.92±7.86c	124.67±2.17c	107.49±8.62c	85.12±6.77b	54.92±1.47b	811.54±27.87c
21	2963.44±58.66d	185.10±2.05d	208.53±25.45c	196.33±11.87d	178.21±11.10d	155.76±8.43d	105.41±5.61c	57.96±4.84b	1120.61±24.61d

2 Values are mean±SE (n=4 for aboveground plant residue, and n=4 for root biomass). Significant differences between different stand ages at the  
 3 same soil layers are indicated by different letters at *P*=0.05

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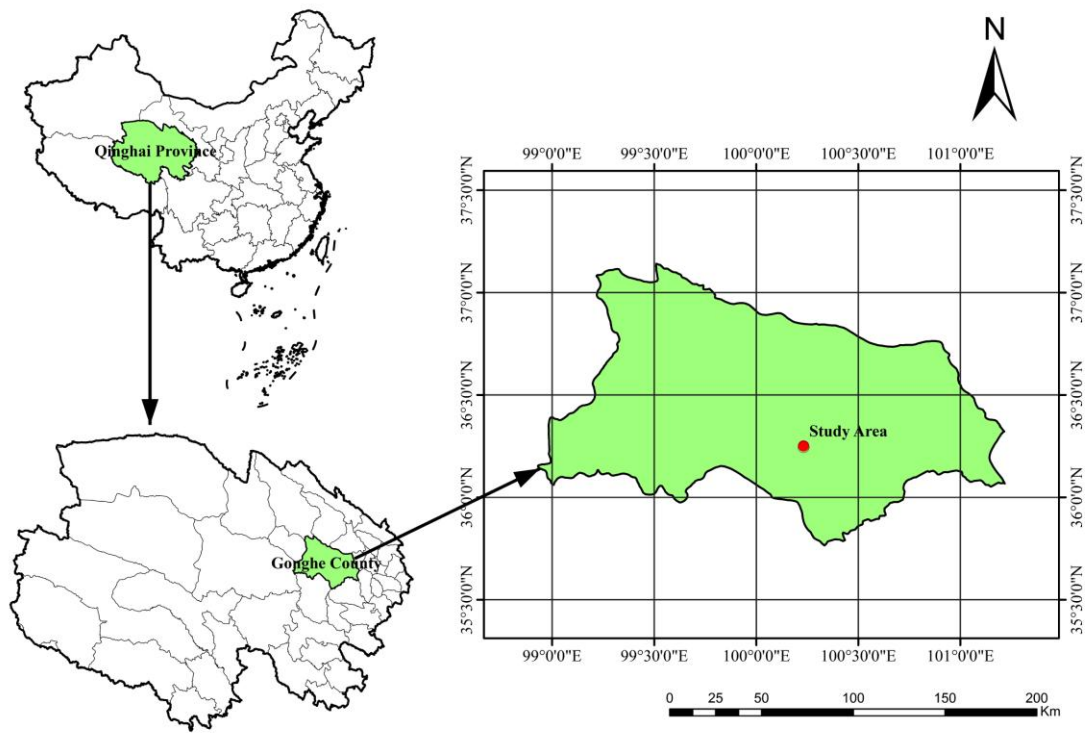
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1 Table 3. Gains and losses of soil organic carbon (SOC) and total nitrogen (TN) at different  
 2 stands relative to the 0-year stand

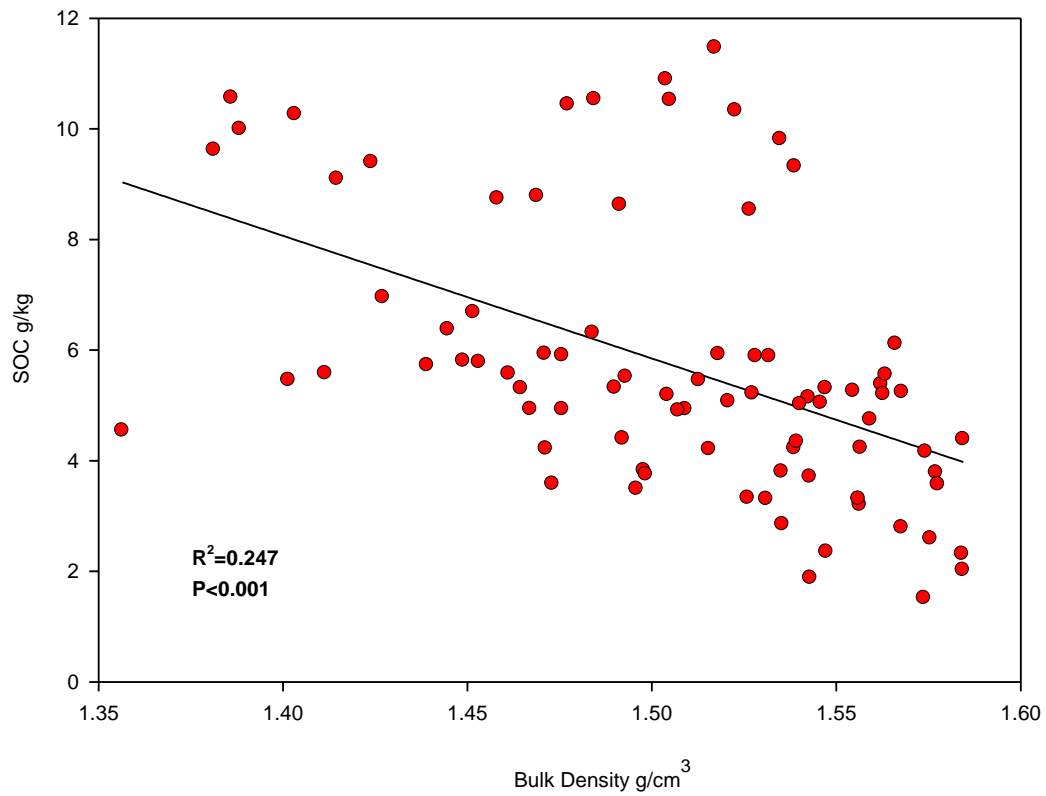
Depth /cm	6a		11a		16a		21a	
	Mass (Mg ha <sup>-1</sup> )	%	Mass (Mg ha <sup>-1</sup> )	%	Mass (Mg ha <sup>-1</sup> )	%	Mass (Mg ha <sup>-1</sup> )	%
SOC								
0-10	0.32	53.87	1.13	80.3	1.32	82.63	1.34	82.81
10-20	0.18	18.79	0.01	1.44	0.57	42.39	0.61	43.99
20-30	0.21	31.01	-0.02	-4.50	0.35	42.97	0.82	63.53
30-50	0.44	38.26	0.56	44.12	0.75	51.28	1.00	58.46
50-100	1.66	59.24	2.21	66.00	2.56	69.18	3.11	73.19
100-150	0.41	25.58	1.99	62.45	2.78	69.89	2.93	71.00
150-200	0.67	38.42	1.93	64.15	2.99	73.50	3.24	75.05
0-200	3.89	40.82	7.82	58.06	11.32	66.71	13.05	69.79
TN								
0-10	0.09	16.49	0.14	23.24	0.26	36.48	0.54	54.20
10--20	0.06	11.78	0.09	16.11	0.10	17.89	0.30	38.62
20-30	0.02	4.29	0.05	8.81	0.14	20.51	0.34	39.05
30-50	0.05	6.15	0.26	24.05	0.46	36.40	0.93	53.45
50-100	0.34	15.61	0.58	23.81	0.89	32.42	1.72	48.12
100-150	0.22	10.28	0.53	21.55	0.77	28.45	1.25	39.29
150-200	0.20	9.36	0.33	14.50	0.68	25.77	0.37	15.99
0-200	1.00	11.06	1.98	19.80	3.30	29.18	5.45	40.47



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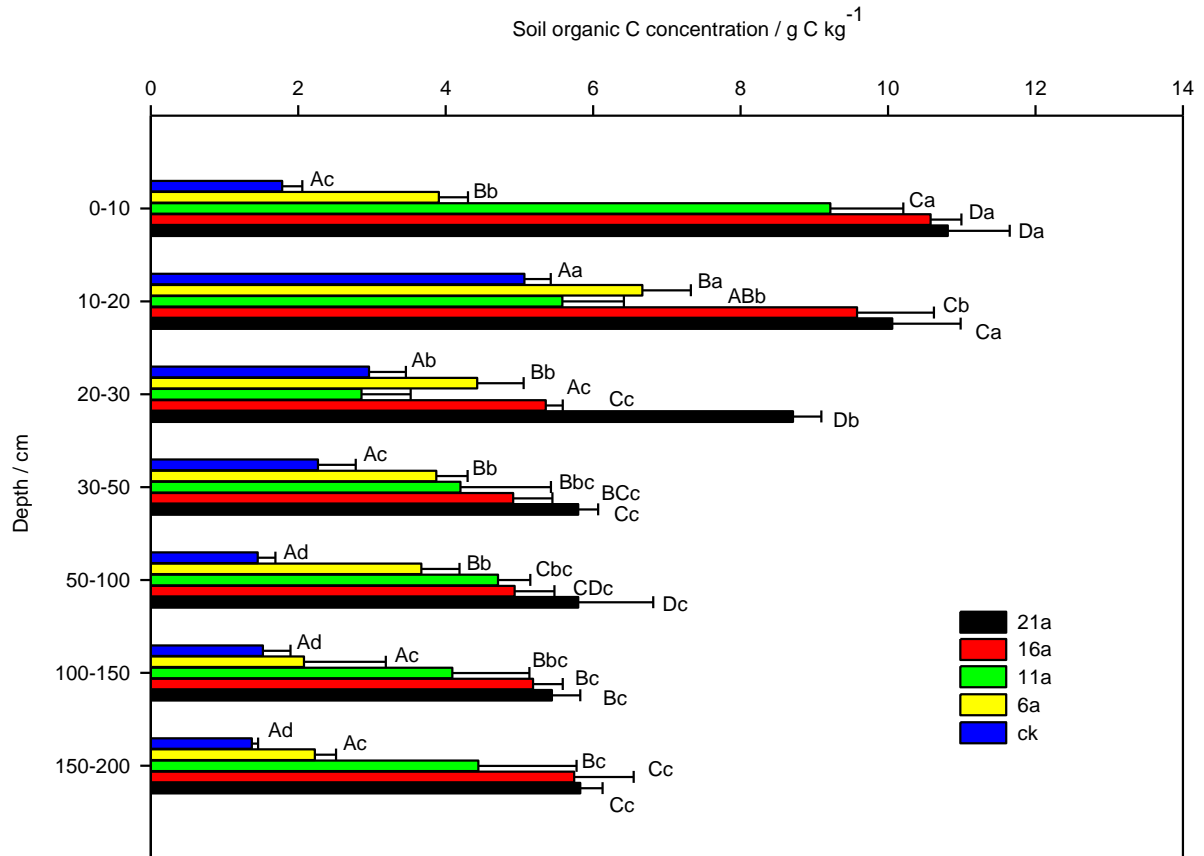
3 Figure 1. Location of the study area, Gonghe County, Qinghai Province, China.



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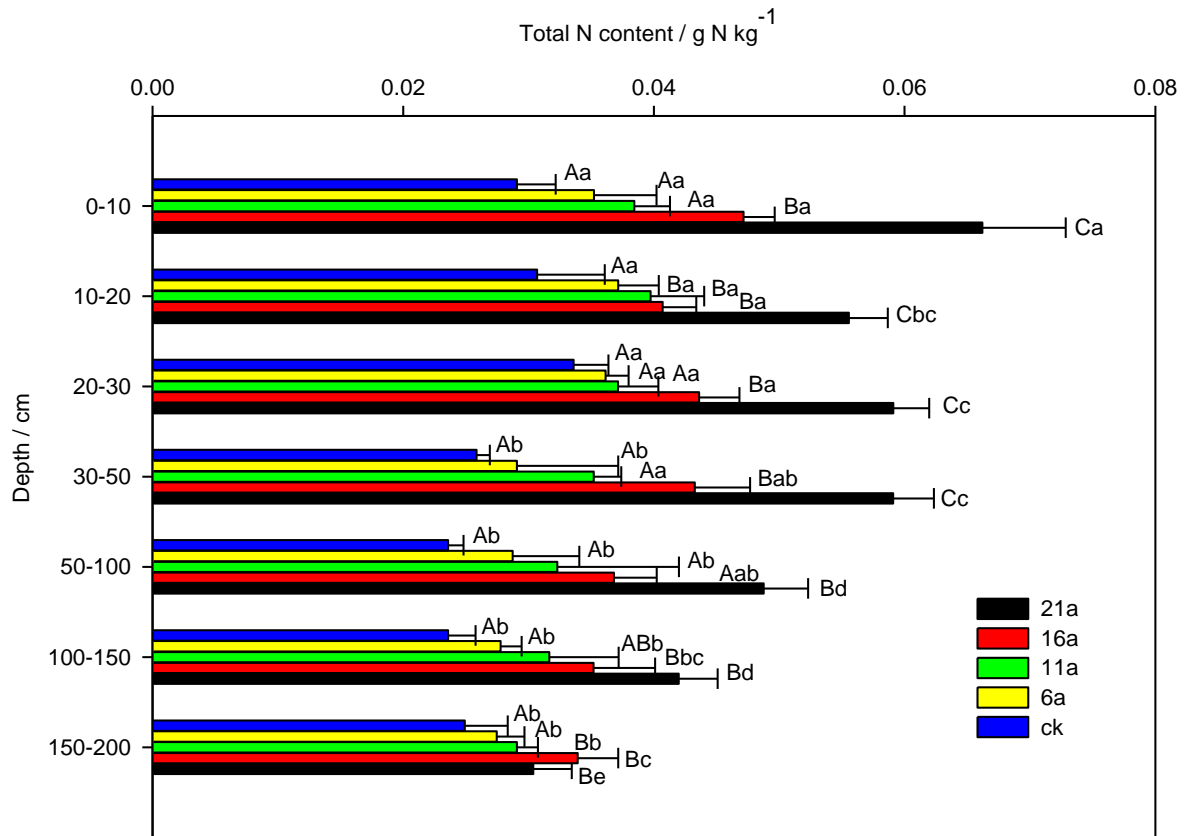
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3 Figure 2. The relationship between soil organic carbon (SOC) and bulk density of *S.*  
4 *cheilophila*.



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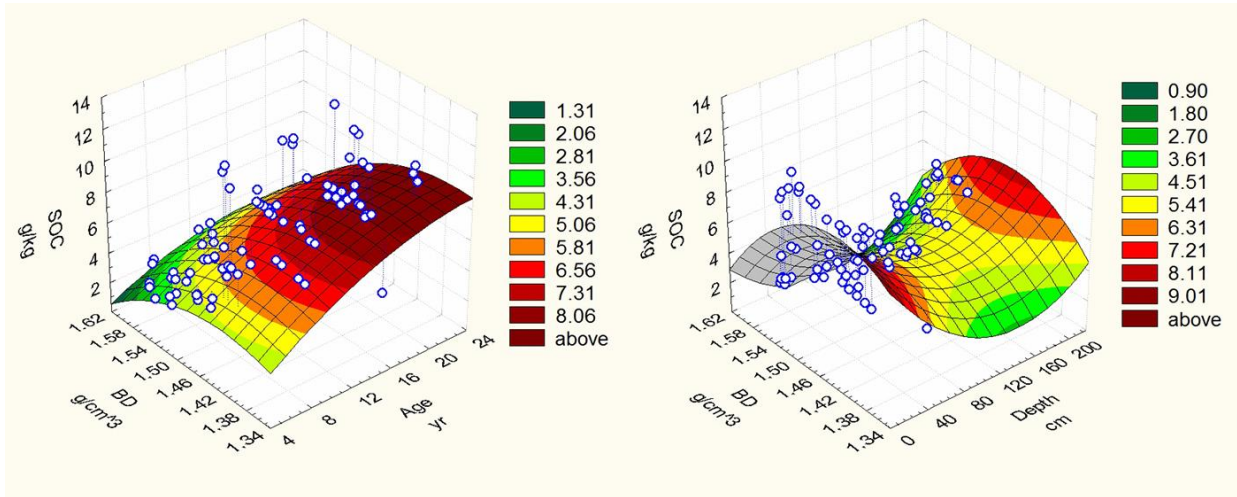
3 Figure 3. Variations in soil organic carbon concentration at different soil depths in different  
 4 stand ages. Values are means  $\pm$ SE. Different uppercase letters indicate significant differences  
 5 in different stand ages, different lowercase letters indicate significant differences at different  
 6 soil depths ( $P < 0.05$ ).



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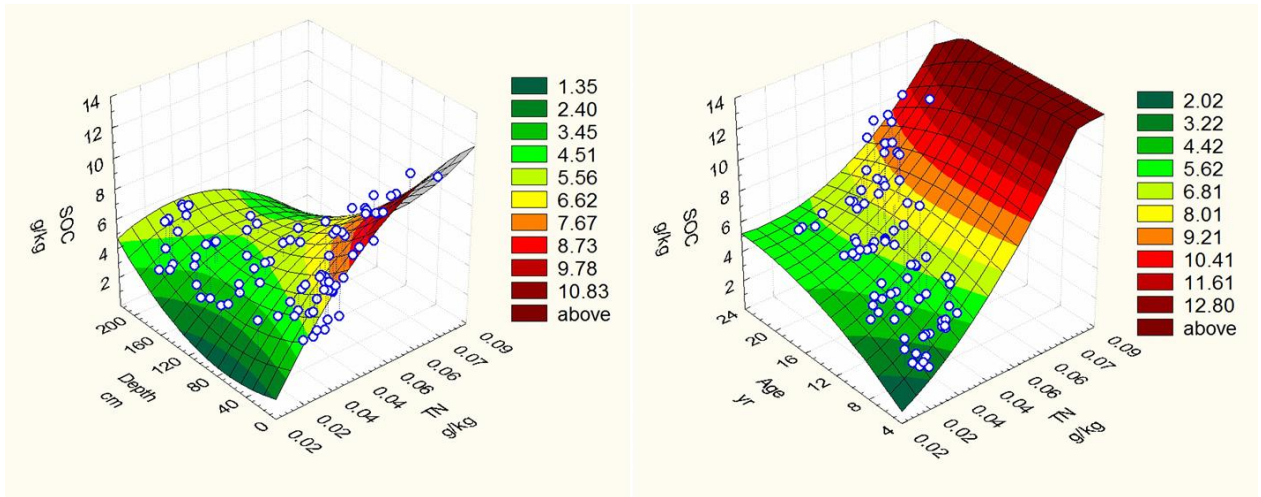
3 Figure 4. Variations in total nitrogen (Total N) content at different soil depths in different  
 4 stand ages. Values are means  $\pm$ SE. Different uppercase letters indicate significant differences  
 5 in different stand ages, different lowercase letters indicate significant differences at different  
 6 soil depth ( $P < 0.05$ ).



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3 Figure 5. Regression models of soil organic carbon (SOC) and bulk density (BD) with  
 4 extension of stand age and soil depth.



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3 Figure 6. Regression models of soil organic carbon (SOC) and total nitrogen(TN) with the  
 4 extension of stand age and soil depth.