

# Soil aggregate stability and aggregate-associated carbon and nitrogen in natural restoration grassland and Chinese red pine plantation on the Loess Plateau



Guang-yu Zhu <sup>a</sup>, Zhou-ping Shangguan <sup>a,b</sup>, Lei Deng <sup>a,b,\*</sup>

<sup>a</sup> State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, Yangling, Shaanxi 712100, PR China

<sup>b</sup> Institute of Soil and Water Conservation, Chinese Academy of Sciences and Ministry of Water Resources, Yangling, Shaanxi 712100, PR China

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## ABSTRACT

Artificial afforestation and natural recovery from abandoned cropland are two typical recovery types on the Loess Plateau, China. However, few studies have investigated the difference of natural secondary vegetation restoration and man-made plantation in soil aggregate physicochemical properties and soil aggregate stability. Therefore, we have selected natural restoration grassland and Chinese red pine plantation to study the differences of soil aggregate size distributions, aggregate carbon (C) and nitrogen (N) distributions, soil aggregate stability index (fractal dimension, *D*; mean weight diameter, MWD; geometric mean diameter, GMD; percentage of aggregation destruction, PAD) as well as their relationships. The results showed that after ~15 years restoration from abandoned cropland, natural restoration grassland had higher soil organic carbon (SOC), total nitrogen (TN), ammonium nitrogen (AN), microbial biomass nitrogen (MBN) and MWD compared to Chinese red pine forest, but Chinese red pine forest had higher aggregate C and N, *D*, GMD and PAD. In addition, SOC positively correlated with MWD in natural restoration grassland but opposite in Chinese red pine forest. In detail, the differences of soil general properties and aggregate size fraction percentages between two land use types were found mainly in 2–5 mm, 1–2 mm, 0.25 mm and clay water-stable aggregate size fractions. The results suggested that higher C content would further contribute the soil aggregate stability in natural restoration grassland, and higher N content would be more important in Chinese red pine plantation.

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## 1. Introduction

For terrestrial ecosystems, land use changes probably will have the largest effect, followed by climate change, nitrogen (N) deposition, biotic exchange and elevated carbon dioxide concentration (Sala et al., 2000). Human beings face the growing challenge of managing trade-offs between immediate human needs and maintaining the capacity of the biosphere to provide goods and services due to irrational land uses (Foley et al., 2005; Smith et al., 2016). China's "Grain for Green" Program, which has launched since 1999, focused on local environment restoration by planting trees in semi-arid regions and by protecting natural recovery (Deng et al., 2014a, 2014b). Over the past decade, land use and vegetation types on the semi-arid Loess Plateau have changed

significantly, converting cropland to other land uses, such as artificial grassland, shrub land, forest or abandoned cropland (Liu et al., 2014).

Land use changes, especially of abandoned cropland may rapidly change soil quality (Deng et al., 2013, 2014a, 2014b; Deng and Shangguan, 2016). Several studies have demonstrated that vegetation restoration could significantly enhance the soil organic carbon (SOC), N content and soil aggregate stability (An et al., 2010; Jiao et al., 2012; Raiesi, 2012; Deng et al., 2016; Deng and Shangguan, 2016), and increase mean weight diameter (Liu et al., 2014), soil fractal dimension and geometric mean diameter (Zhuang et al., 2012). In addition, soil aggregate size distribution and stability are important indicators of soil physical quality (Castro Filho et al., 2002; Shrestha et al., 2007). As we know, soil organisms and soil chemistry had a causal relation with soil physical quality. Jin et al. (2015) have indicated soil structure losses in soils due to lower SOC inputs, this result was the same with many studies (Pagliai et al., 2004; Haynes, 2005). Plant microbial symbionts also played a significant role in aggregate stability (Hosseini et al., 2015). Meanwhile, soil aggregation may be determined by the mean weight diameter (MWD), the geometric mean diameter (GMD) (Castro Filho et

\* Corresponding author at: State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, Northwest A&F University, NO. 26 Xinong Road, Yangling, Shaanxi 712100, PR China.

E-mail address: [leideng@ms.iswc.ac.cn](mailto:leideng@ms.iswc.ac.cn) (L. Deng).

al., 2002), soil fractal dimension (D) (Ahmadi et al., 2011) and the percentage of aggregation destruction (PAD) (Yu et al., 2013).

In the Loess Plateau, due to the implementation of “Grain for Green” Program, artificial afforestation and natural recovery from abandoned cropland are two typical recovery methods on the Loess Plateau. Recent research mainly focused on the effects of nutrient addition on soil aggregate stability and soil C and N cycles (Martens, 2000; Mizuta et al., 2015; Mohanty et al., 2015; Wang et al., 2016a), the effects of land uses on aggregation and SOC and N fractions and soil aggregate stability (Huang et al., 2010; Liu et al., 2014; Li et al., 2016), and the effects of soil chronosequence on SOC and soil quality (An et al., 2008; Duchicela et al., 2013; Deng et al., 2014a, 2016). In addition, several studies also focused on the relationship between soil physicochemical properties and soil aggregate stability (Six et al., 2004; Sarathjith et al., 2014; Chaplot and Cooper, 2015). However, few studies have investigated the difference of two land uses (natural restoration grassland and man-made Chinese red pine forest) in soil aggregate physicochemical properties and soil aggregate stability.

In this study, we examined the effect of natural restoration grassland and Chinese red pine plantation on soil physicochemical properties in Ziwuling Forest Farm on the Loess Plateau, China. The objectives of this study were: (1) to determine the difference of dry sieving and wet sieving soil aggregate size distributions and wet sieving aggregate-associated C and N distributions in two land use types, (2) to make clear the differences of soil aggregate stability index (D, MWD, GMD, PAD) of dry sieving and wet sieving in two land use types, and (3) to find the relationships between soil aggregate stability index and its soil physicochemical properties in the two land use types on Loess Plateau. We expected that the reasonable land use type will be predicted in this area.

## 2. Materials and methods

### 2.1. Study area

The experimental site was managed on the Lianjiabian Forest Farm of the Heshui County, Gansu Province, China (108°10′–109°18′E, 35°03′–36°07′N), located in the hinterland of the Loess Plateau. The Ziwuling forest region, covering a total area of 23 km<sup>2</sup>. The altitude of the region's hilly and gully land-forms is 1211–1453 m.a.s.l., their relative height difference is about 200 m, the area's mean annual temperature is 10 °C, mean annual rainfall is 587 mm (Deng et al., 2013, 2016; Wang et al., 2016b), accumulative temperature is 2761 °C, and the annual frost-free period is 112–140 days. Soils of this region are largely loessial, having developed from primitive or secondary loess parent materials, which are evenly distributed at thicknesses of 50–130 m above red earth consisting of calcareous cinnamon soil. The artificial communities throughout the region are *Pinus tabulaeformis* (Carr.), *Hippophae rhamnoides* (Linn.) and *Robinia pseudoacacia* (Linn.). These forests canopy density ranging between 80% and 90%.

In the Ziwuling forest region, duo to China's government launched ‘Grain to Green Program’ to plant trees and protect natural recovery since 1999, the Lianjiabian Forest Farm had planted many Chinese red pine forest on abandoned farmlands, meanwhile, lots of farmlands had been abandoned. Under the background of ‘Grain to Green Program’ implementation, many Chinese red pine forest and natural restoration grasslands were distributed in the study area. Through ~15 years vegetation restoration, the canopy density of the Chinese red pine is about 85% and coverage of the natural grassland is about 75%. *Bothriochloa ischaemum* (Linn.) Keng, *Carex lanceolate* Boott, *Potentilla chinensis* (Ser) and *Stipa bungeana* Trin are the main grassland species. Chinese red pine forest is pure forest and has a high canopy density. In addition, the surface soil of Chinese red pine forest is covered by pine needles, therefore, the single undergrowth plant in Chinese red pine forest mainly are *C lanceolate* Boott, *Hippophae rhamnoides* (Linn.) seedlings and *Pinus tabulaeformis* (Carr.) seedlings.

### 2.2. Samplings and measurements

#### 2.2.1. Experimental design and sampling

In this study, we had used a paired design. Each Chinese red pine forest stand was paired with adjacent natural restoration grassland to ensure the two restoration types had similar land use history (abandoned cropland). The two land use types scattered embedded in the study area. Generally, there have 2000–5000 m<sup>2</sup> area for each vegetation patch. In August 2015, we randomly set up five 20 m × 20 m plots in a ~15 years man-made planting Chinese red pine forest and a ~15 years natural restoration grassland to collect soil samples, respectively in each of the five sites. They were both converted from abandoned cropland. Each paired site is of similar physiographic conditions and slope gradients, and the two plots of each pair are separated 10–20 m. Within the center and four corners of each plot, five 1 m × 1 m quadrats were chosen to sample soils, in total, there are 25 quadrats for each vegetation type. Meanwhile, soil bulk density and undisturbed soil samples were obtained in a random selection of digging pit from each plot.

In each quadrat, soil sampling, done in five soil layers: 0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm and 80–100 cm, was using a soil drilling sampler, and 5 samples were taken from the center and four corners of each plot and mixed to form a bulk sample of about 2 kg for the measurement of soil physical and chemical properties. Each soil was sieved (2 mm) to remove large roots, stones and the macrofauna, and determination of MBC and MBN need soils stored on ice bags. Soil bulk density was measured using a soil bulk sampler with a 5 cm diameter and a 5 cm high stainless steel cutting ring (3 replicates) at 0–20 cm, 20–40 cm, 40–60 cm, 60–80 cm and 80–100 cm soil layers. The original volume of each soil core and its dry mass after oven-drying at 105 °C were measured. In addition, three undisturbed soil samples were taken for aggregate stability analysis at 0–20, 20–40, 40–60, 60–80 and 80–100 cm soil layers from each plot, sealed in lunch box and transported to the laboratory, where they were air dried at room temperature.

#### 2.2.2. Soil physical and chemical properties

Chemical analysis was performed on soil samples at 2 mm, ammonium nitrogen (AN) and nitrate nitrogen (NN) analysis were performed on soil samples at 1 mm, using standard methods. Soil pH and ion exchange (EC) were determined using the method of acidity agent (soil-water ration of 1:5) (PHS-3C pH acidometer, China) and a DDS-307 model Electric Conductivity Detector (Lei-ci, China), respectively. Soil organic carbon (SOC) content was determined by the K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>–H<sub>2</sub>SO<sub>4</sub> oxidation method (Nelson and Sommers, 1996). Soil total nitrogen (TN) content was assayed using the Kjeldahl method (Bremner, 1996). Inorganic or mineral N in soil was extracted by shaking samples in 1 mol L<sup>-1</sup> KCl [1:5(w/w) soil: KCl solution] for 1 h and subsequent filtering through filter paper (Bremner and Keeney, 1966). The filtrate was analyzed for NH<sub>4</sub><sup>+</sup>-N (Crooke and Simpson, 1971) and NO<sub>3</sub><sup>-</sup>-N (Best et al., 1976) with a Chemlab Auto-Analyzer. Soil microbial biomass C (MBC) and N (MBN) were determined using the fumigation-extraction method (Brookes et al., 1985; Wu et al., 1990).

BD was calculated depending on the inner diameter of the core sampler, sampling depth and the oven dried weight of the composite soil samples. Soil water content was measured gravimetrically and expressed as a percentage of soil water to dry soil weight. A laser particle analyzer that operates over a range of 0.02–2000 μm (Mastersizer 2000 particle size analyzer, Malvern Instruments, Ltd., UK) and based on the laser diffraction technique was used to measure particle size.

Aggregates were separated following the modified method described by Six et al. (1998). An air-dried bulk soil consisting of aggregates of diameter equal or lesser than 10 mm was fractionated into 10 mm, 7 mm, 5 mm, 3 mm, 2 mm, 1 mm, 0.5 mm and 0.25 mm classes. In a proportional manner, 50 g of these aggregate fractions was then transferred to a set of five stacking sieves with openings of 5 mm, 2 mm, 1 mm, 0.5 mm and 0.25 mm, resulting in the collection of five aggregate size fraction: >5 mm, 2–5 mm, 1–2 mm, 0.5–1 mm, 0.25–

0.5 mm and <0.25 mm. The sieves were immersed in distilled water and were oscillated for 5 min at a movement of approximately 6 cm at 30 cycles/min. All fractions were dried at 70 °C prior to weighing. The data were analyzed to compute percent of aggregate destruction (PAD), fractal dimension (D) (Tyler and Wheatcraft, 1992), geometric mean diameter (GMD) and mean weight diameter (MWD) (Youker and McGuinness, 1957).

### 2.3. Statistical analysis

T-test was performed to test the differences in all soil properties between the two land use types. Two-way ANOVA was performed to test the differences in land use type, soil layers and the interaction of soil layer and land use type. Significant differences were evaluated at the 0.05 level. Pearson's test was adopted to determine whether there were significant correlations between soil chemical properties and soil physical properties. In addition, we used SPSS to test the autocorrelation of all relationships between soil properties and soil aggregate size fractions in the two land uses, and the conclusion is "dl < DW < du". This result indicated that no significant autocorrelation, so we do not consider autocorrelation. In addition, all processed data were converted into images using the SigmaPlot software program, ver. 12.5 (2011; Systat Software, Inc., leadtools, dundas software ltd., wpcubed GmbH, Germany, TE Sub Systems, Inc., and Sax Software). D, MWD, GMD and PAD were calculated by soil dry-sieved and wet-sieved aggregate size fractions (Appendix Table S1 and Appendix Table S2). All of the statistical analyses were performed using the SPSS software program, ver. 22.0 (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Soil general properties

Compared with Chinese red pine forest, natural restoration grassland exhibited significantly greater SOC, MBN in 0–20 cm soil depth (Fig. 1, a and f), TN, AN in 20–80 cm soil depths (Fig. 1, b and d) and BD in 20–40 cm soil depth (Fig. 1, l). However, MBC in 0–20 cm soil depth (Fig. 1, e), C/N in 20–80 cm soil depths (Fig. 1, h), pH (P < 0.01)

in 0–40 cm soil depths (Fig. 1, i), EC (P < 0.0001) in 80–100 cm soil depths (Fig. 1, j), SW (P < 0.01) in 0–20 cm and 60–100 cm soil depths (Fig. 1, k) and BD in 60–100 cm soil depth (Fig. 1, l) in Chinese red pine forest were significantly greater than in natural restoration grassland.

### 3.2. Soil aggregate stability

The D of the wet-sieved aggregates at 0–20 cm and 40–60 cm soil depths in Chinese red pine forest was significantly higher than in natural restoration grassland, and the D of the dry-sieved aggregates (P < 0.01) at 0–40 cm soil depths in Chinese red pine forest was significantly higher than that in natural restoration grassland (Fig. 2, a). Chinese red pine forest had significantly increased the MWD of the wet-sieved aggregates in 0–40 cm soil depths and the MWD of the dry-sieved aggregates (P < 0.01) in 0–40 cm and 60–100 cm soil depths (Fig. 2, b). The GMD, calculated by wet and dry sieved aggregate size fractions at 0–20 cm soil depth (P < 0.0001) was significantly higher in Chinese red pine forest than that in natural restoration grassland (Fig. 2, c). Meanwhile, Chinese red pine forest had significantly decreased the GMD of wet-sieved aggregates in 40–60 cm and 80–100 cm soil depths (Fig. 2, c). Natural restoration grassland had significantly increased PAD in 20–60 cm and 80–100 cm soil depths (Fig. 2, d).

### 3.3. Carbon, nitrogen and phosphorus associated with aggregate size fractions

The >5 mm (P < 0.01), 5–2 mm aggregate SOC contents had significant difference at the depths of 80–100 cm between Chinese red pine forest and natural restoration grassland (Table 1). At these soil depths, the aggregate SOC contents under Chinese red pine forest were significantly greater than under natural restoration grassland. Meanwhile, 1–5 mm, 0.5–0.25 mm (P < 0.01) and <0.25 mm aggregate SOC contents at 0–20 cm and 20–40 cm soil depths also followed this trend (Table 1). The >5 mm, 1–0.5 mm and 0.5–0.25 (P < 0.01) mm aggregate nitrogen content at 0–20 cm and 20–40 cm soil depths in Chinese red pine forest were significantly greater than in natural restoration grassland. Chinese red pine forest had significantly increased the 5–2 mm aggregate nitrogen content in 20–80 cm soil depths (Table 1). However, there were no

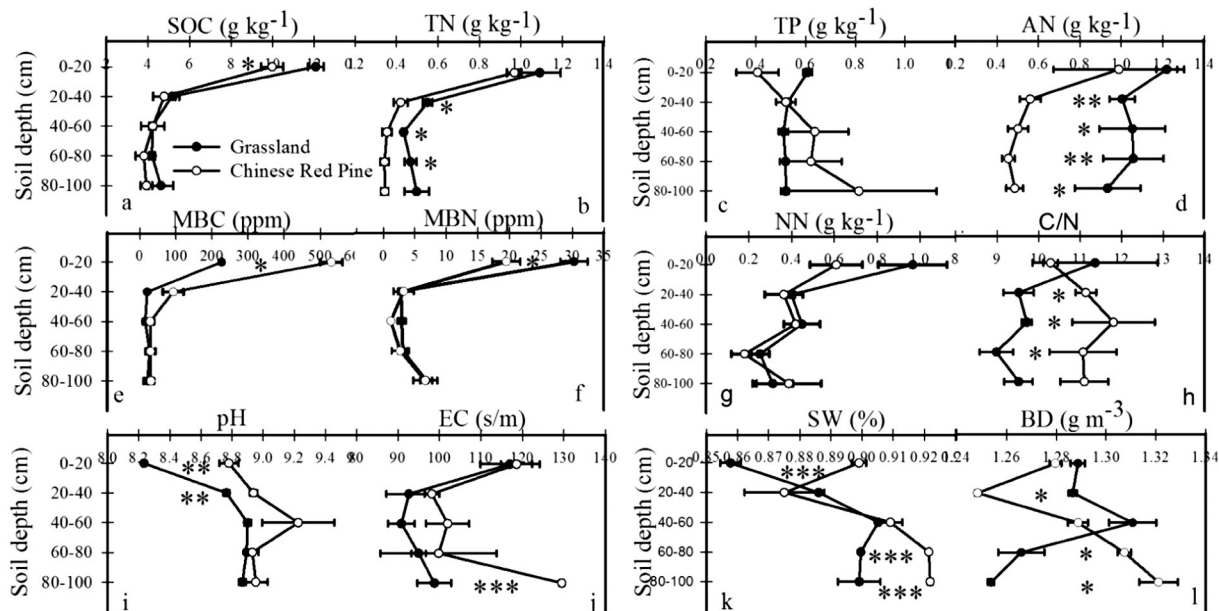
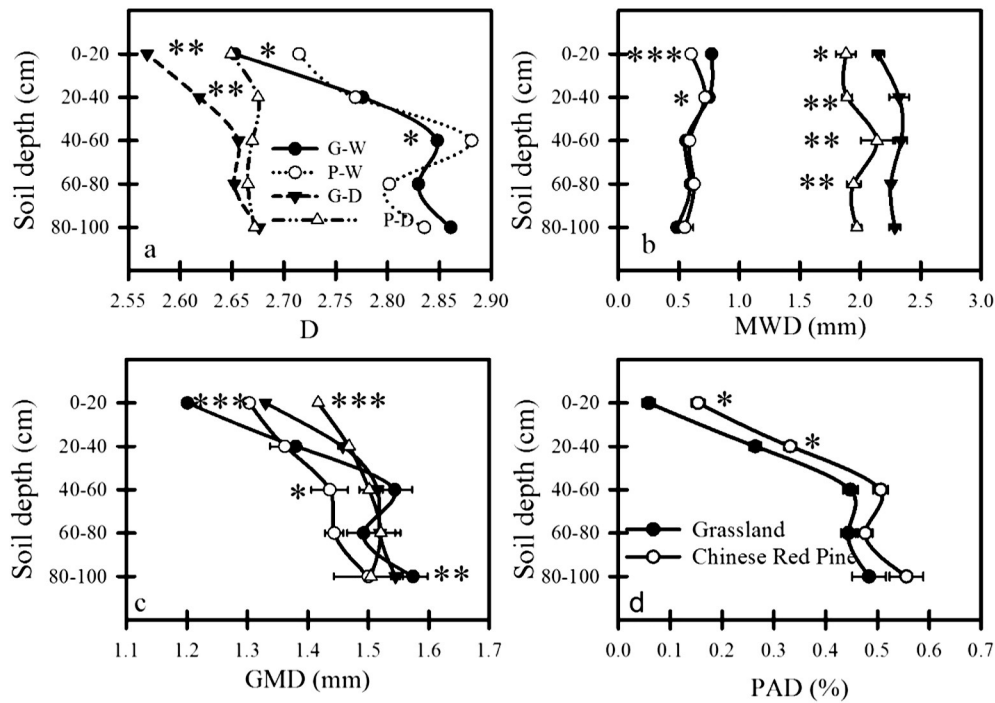


Fig. 1. Effect of natural restoration grassland and Chinese red pine forest on (a) SOC, (b) TN, (c) TP, (d) AN, (e) MBC, (f) MBN, (g) NN, (h) C/N, (i) pH, (j) EC, (k) SW and (l) BD. The values are Mean  $\pm$  SE, and the sample size  $n = 5$ . Significant differences between fenced and grazed communities are indicated by symbols as follows: \*\*\*P < 0.001, \*\*P < 0.01, \*P < 0.05.



**Fig. 2.** Effect of natural restoration grassland and Chinese red pine forest on (a) D of wet (W) and dry sieved (D), (b) MWD of wet and dry sieved, (c) GMD of wet and dry sieved and (d), PAD. The values are Mean  $\pm$  SE, and the sample size  $n = 5$ . Significant differences between fenced and grazed communities are indicated by symbols as follows: \*\*\* $P < 0.001$ , \*\* $P < 0.01$ , \* $P < 0.05$ . G, grassland; P, Chinese red pine.

significant differences in the 2–1 mm aggregate SOC contents at all soil depths, 2–1 mm and  $<0.25$  mm nitrogen content is the same (Table 1). Overall, SOC and TN were mainly distributed in the large aggregate.

### 3.4. Relationships between soil properties and aggregate size fractions

Water-stable aggregate size fractions of 2–5 mm, 1–2 mm, and  $<0.25$  mm were the main difference in natural restoration grassland

and Chinese red pine forest (Table 2). Clay and sand also had a difference in the two land use types (Table 2). The different relationships between soil chemical properties and their aggregate size fractions in the two land use types were presented in three ways (Table 2): natural restoration grassland had a significant relationship and Chinese red pine forest had not (e.g. SOC in all soil aggregate size fractions), Chinese red pine forest had a significant relationship and natural restoration grassland had not (e.g. AN in all soil aggregate

**Table 1**  
SOC and TN content in water-stable aggregates in different aggregate size classes of soil from different land uses of natural restoration grassland and Chinese red pine forest. Values are in the form of the Mean  $\pm$  SE. Values are the sample size  $n = 5$ .

Land use types	Soil depth (cm)	Aggregate size					
		$>5$ mm	5–2 mm	2–1 mm	1–0.5 mm	0.5–0.25 mm	$<0.25$ mm
Grassland-SOC	0–20	10.89 $\pm$ 0.90	11.06 $\pm$ 0.40	10.42 $\pm$ 0.65	10.50 $\pm$ 0.35 A	10.39 $\pm$ 0.35B	8.71 $\pm$ 0.16 A
	20–40	3.95 $\pm$ 0.11	4.04 $\pm$ 0.21	3.58 $\pm$ 0.16	3.86 $\pm$ 0.19 A	3.61 $\pm$ 0.29	3.16 $\pm$ 0.24 A
	40–60	3.48 $\pm$ 0.26	3.49 $\pm$ 0.07	3.37 $\pm$ 0.06	3.01 $\pm$ 0.06	3.28 $\pm$ 0.09	2.85 $\pm$ 0.10
	60–80	3.36 $\pm$ 0.14	3.66 $\pm$ 0.21	3.25 $\pm$ 0.22	3.09 $\pm$ 0.22	3.24 $\pm$ 0.17	2.85 $\pm$ 0.19
	80–100	3.35 $\pm$ 0.18B	3.60 $\pm$ 0.12 A	4.64 $\pm$ 1.24	3.16 $\pm$ 0.10	3.24 $\pm$ 0.07	2.81 $\pm$ 0.15
Chinese red pine forest -SOC	0–20	10.97 $\pm$ 1.03	11.32 $\pm$ 0.65	12.38 $\pm$ 0.34	12.36 $\pm$ 0.47 A	12.27 $\pm$ 0.99B	10.18 $\pm$ 0.62 A
	20–40	4.35 $\pm$ 0.22	4.03 $\pm$ 0.11	4.09 $\pm$ 0.21	4.57 $\pm$ 0.22 A	4.01 $\pm$ 0.25	4.00 $\pm$ 0.34 A
	40–60	3.79 $\pm$ 0.15	3.67 $\pm$ 0.14	3.19 $\pm$ 0.10	3.20 $\pm$ 0.07	3.15 $\pm$ 0.10	2.70 $\pm$ 0.08
	60–80	3.63 $\pm$ 0.10	4.01 $\pm$ 0.27	3.21 $\pm$ 0.07	3.38 $\pm$ 0.09	2.96 $\pm$ 0.32	2.98 $\pm$ 0.30
	80–100	4.83 $\pm$ 0.35B	4.17 $\pm$ 0.18 A	3.50 $\pm$ 0.22	3.67 $\pm$ 0.29	3.03 $\pm$ 0.22	2.84 $\pm$ 0.27
Grassland-N	0–20	1.02 $\pm$ 0.18	1.00 $\pm$ 0.14	1.15 $\pm$ 0.05	1.14 $\pm$ 0.06 A	1.09 $\pm$ 0.03B	1.02 $\pm$ 0.04
	20–40	0.49 $\pm$ 0.07 A	0.49 $\pm$ 0.03 A	0.51 $\pm$ 0.05	0.41 $\pm$ 0.03 A	0.41 $\pm$ 0.04B	0.44 $\pm$ 0.04
	40–60	0.38 $\pm$ 0.02 A	0.36 $\pm$ 0.01B	0.36 $\pm$ 0.01	0.32 $\pm$ 0.01 A	0.33 $\pm$ 0.01	0.33 $\pm$ 0.02
	60–80	0.44 $\pm$ 0.06	0.34 $\pm$ 0.00C	0.32 $\pm$ 0.01	0.39 $\pm$ 0.03	0.32 $\pm$ 0.01	0.29 $\pm$ 0.02B
	80–100	0.36 $\pm$ 0.02	0.37 $\pm$ 0.01	0.35 $\pm$ 0.01	0.36 $\pm$ 0.02	0.33 $\pm$ 0.02	0.29 $\pm$ 0.01
Chinese red pine forest -N	0–20	1.19 $\pm$ 0.03	1.20 $\pm$ 0.05	1.24 $\pm$ 0.03	1.32 $\pm$ 0.04 A	1.28 $\pm$ 0.01B	1.10 $\pm$ 0.01
	20–40	0.77 $\pm$ 0.05 A	0.58 $\pm$ 0.03 A	0.61 $\pm$ 0.04	0.55 $\pm$ 0.05 A	0.61 $\pm$ 0.02B	0.60 $\pm$ 0.06
	40–60	0.41 $\pm$ 0.04 A	0.46 $\pm$ 0.03B	0.41 $\pm$ 0.02	0.44 $\pm$ 0.05 A	0.35 $\pm$ 0.01	0.35 $\pm$ 0.01
	60–80	0.43 $\pm$ 0.07	0.40 $\pm$ 0.01C	0.44 $\pm$ 0.07	0.36 $\pm$ 0.02	0.46 $\pm$ 0.10	0.39 $\pm$ 0.02B
	80–100	0.39 $\pm$ 0.01	0.41 $\pm$ 0.03	0.37 $\pm$ 0.01	0.44 $\pm$ 0.09	0.35 $\pm$ 0.01	0.32 $\pm$ 0.00

Note: Significant differences between natural restoration grassland and Chinese red pine forest are indicated by capital letter: "C"  $P < 0.001$ , "B"  $P < 0.01$ , "A"  $P < 0.05$ .

**Table 2**

Pearson's correlations between soil aggregate size fractions, soil particle size and soil properties in five soil layers at soil depth of 0–100 cm (n = 25).

Land use types	Soil property	Aggregate size						Soil particle	
		>5 mm	2–5 mm	1–2 mm	0.5–1 mm	0.25–0.5 mm	<0.25 mm	Clay (%)	Sand (%)
Grassland	SOC	0.562**	0.506**		–0.523**	–0.540**	–0.517**		
	TN	0.921**	0.740**	0.478**	–0.786**	–0.884**	–0.847**		
	C/N	–0.625*			0.538*	0.631*	0.589*		
	MBC	0.521*			–0.516*	–0.530*			
	MBN	0.705**	0.627*		–0.650**	–0.715**			
	pH	–0.745**	–0.665**		0.707**	0.747**	0.704**		
	SW	–0.797**	–0.637*		0.849**	0.864**	0.706**		
	MWDW	0.722**	0.960**	0.943**		–0.571**	–0.874**		
	GMDW	–0.911**	–0.957**	–0.807**	0.494**	0.725**	0.988**		
	PAD	–0.956**	–0.960**	–0.751**	0.602**	0.801**	0.999**		
Chinese red pine forest	SOC							–0.524*	0.561*
	TN	0.753**		–0.903**	–0.889**	–0.959**	0.925**	0.771**	
	AN	0.578**			–0.539*	–0.502*	–0.541*	0.485*	
	C/N	–0.870**	–0.737**		0.727**	0.847**	0.846**	–0.710**	
	MBC	0.717**		–0.737**	–0.697**	–0.585*	–0.669**	0.637*	
	MBN	0.734**		–0.772**	–0.709**	–0.590*	–0.690**	0.654**	
	pH			0.504*	0.571**				
	SW		–0.691**			0.559*			
	MWDW		0.723**	0.719**					
	GMDW	–0.746**	–0.851**			0.597**	0.901**	–0.577**	
PAD	–0.955**	–0.874**		0.773**	0.831**	0.998**	–0.705**		

\* Correlation significant at the 0.05 level (2-tailed).

\*\* Correlation significant at the 0.01 level (2-tailed).

size fractions), natural grassland and Chinese red pine forest had an inverse relationship (e.g. TN in 1–2 mm and <0.25 mm aggregate fractions).

**Table 3**

Two-way ANOVA results for the effects of land use type, soil layer and their interaction on soil properties and soil particle size.

Soil properties	Variance source	Land use type	Soil layer	Land use type × soil layer
		1	4	4
		Degree of freedom		
MBC	F-Value	8.071	93.812	5.452
	P-Value	<b>0.010**</b>	<b>0.000***</b>	<b>0.004**</b>
MBN	F-Value	72.392	215.537	36.796
	P-Value	<b>0.000***</b>	<b>0.000***</b>	<b>0.000***</b>
AN	F-Value	25.408	2.443	0.501
	P-Value	<b>0.000***</b>	0.073	0.735
NN	F-Value	1.743	9.036	1.310
	P-Value	0.199	<b>0.000***</b>	0.293
TN	F-Value	17.541	80.879	0.138
	P-Value	<b>0.000***</b>	<b>0.000***</b>	0.966
TC	F-Value	8.118	0.470	1.847
	P-Value	<b>0.009**</b>	0.757	0.153
pH	F-Value	14.745	9.202	2.302
	P-Value	<b>0.001**</b>	<b>0.000***</b>	0.087
EC	F-Value	6.523	5.271	1.427
	P-Value	<b>0.017*</b>	<b>0.003**</b>	0.255
SW	F-Value	19.356	19.905	7.861
	P-Value	<b>0.000***</b>	<b>0.000***</b>	<b>0.001**</b>
BD	F-Value	3.579	1.076	2.081
	P-Value	0.073	0.394	0.121
Clay	F-Value	3.049	2.537	0.923
	P-Value	0.094	0.066	0.467
Silt	F-Value	9.743	1.067	0.175
	P-Value	<b>0.005**</b>	0.395	0.949
Sand	F-Value	9.321	0.531	0.093
	P-Value	<b>0.005**</b>	0.714	0.984

The bold data indicates significant differences in Two-way ANOVA results.

\* Indicates significant at P &lt; 0.05.

\*\* Indicates significant at P &lt; 0.01.

\*\*\* Indicates significant at P &lt; 0.001.

## 4. Discussions

### 4.1. Effects of land use type, soil layer and their interactions on soil general properties

In our study, MBC, MBN and SW were significantly affected by land use type, soil layer and their interactions (Table 3). EC, pH, and TN were not affected by the interaction of land use type and soil layer, but SOC and AN were affected by only land use type. However, Deng et al. (2014a) demonstrated SOC can also be affected by soil layer. The distribution of SOC in soils is influenced by the quality and quantity of inputs determined by the integrated effects of species-specific traits (Schmidt et al., 2011). In this study, because most of grass species are annuals in the natural restoration grassland, meanwhile, natural restoration grassland also have more less root biomass as the input of soil organic matter compared with Chinese red pine forest, especially in deeper soil depth due to the short-term natural recovery. In addition, compared with grassland communities, the lower decomposition constant and more half time of mass loss indicates that pine needles was harder to be decomposed (Osono et al., 2014), which caused the greater soil chemical content in natural restoration grassland (Fig. 1, a, b, d and f). Zeng et al. (2008) also found the same results. However, SOC and N of some soil aggregate fractions in Chinese red pine forest were significantly increased (Table 1). Therefore, soil aggregate chemical properties and soil texture showed that Chinese red pine forest played a stronger role in soil transformation (Table 1 and Appendix Table S2). Wang et al. (2013) observed that MBC: MBN > 20:1 will result in a low biological activity of soil nitrogen, and MBC: MBN > 5:1 will improve biological activity of soil nitrogen and decreased nitrogen loss. In this study, the MBC: MBN of Chinese red pine forest was 26:1, and grassland was 7:1. The greater MBC and lower MBN of Chinese red pine forest (Fig. 1, e and f) primarily because the microorganism in Chinese Pine used a lot of soil N to decompose the pine needles and create a good environment (Fig. 1, i and k). In addition, Xue et al. (2013) reported that AN is more primarily aggregates and more prevalent in the surface soil than in deeper layers, which is the opposite of our findings (Fig. 1, d). Perhaps the higher pH, SW and BD of Chinese red pine forest caused higher AN distributed in deeper soils (Fig. 1, d, i, k and l).

#### 4.2. Distributions of soil aggregate stability index between dry and wet sieve methods in two land use types

Based on researches of Salako et al. (1999) and Tang et al. (2016), D, PAD and MWD demonstrated that surface soil of natural restoration grassland was better than Chinese red pine forest (Fig. 2, a, b and d). Although the distribution of GMD in Chinese red pine forest surface soil was significantly higher than natural restoration grassland, it is reversed in deeper soil depth (Fig. 2, c). The results showed that soil aggregate stability of natural restoration grassland was better than Chinese red pine forest, and considering the soil erosion, natural restoration is a good choice due to GMD in Chinese red pine increased. Bearden and Petersen (2000) demonstrated that the unpasteurized soil had significantly higher GMD than the pasteurized soil because of *Arbuscular mycorrhizal* (AM) fungi. Probably because the Chinese red pine forest surface soil accumulates more pine needles, leading to the condition of high AM fungi in Chinese red pine forest surface soil. Furthermore, compared with Chinese red pine forest, natural restoration grassland input more roots into the deeper soil every year also could cause difference in the distribution of GMD between the two land use types. In addition, D, MWD of wet-sieved was less than dry-sieved, this result indicated that there were lots of dried soil aggregates in this area (Fig. 2, a and b). Therefore, in terms of this results of soil aggregate stability, the area needs ongoing ecological restoration.

#### 4.3. Relationships between soil chemical properties and soil aggregate stability index in two land types

The stability of SOC was influenced by soil aggregation, providing microenvironments of physical protection and absorbing particle organic matter (Fang et al., 2015). In this study, a significant negative correlation between the PAD and SOC and TN of two land use type was found (Table 4). Unlike Su et al.'s (2007) research, the study showed a significant positive correlation between the PAD and SOC in the natural restoration grassland (Table 4). Eynard et al. (2005) indicated that SOC was the important material basis of the formation of soil aggregate, and soil aggregate condition also affected the decomposition of SOC. Fang et al. (2015) also found higher OC concentration in smaller aggregates,

probably because smaller aggregates have larger surface area and then can absorb more OC. On the other hand, decomposition efficiency of microbe and enzyme on OC may be lower in smaller aggregates owing to greater physical protection (Fang et al., 2015). In this study, we found the relationship between SOC and macro-aggregate was not significant in grassland. Perhaps  $\text{CaCO}_3$  was the main cementing material of loess aggregate (Guo et al., 2004). Otherwise, the result of grassland also showed that the more SOC contents has, the more >2 mm soil water-stable aggregate size fractions have, and the less <1 mm soil water-stable aggregate size fractions have (Table 2), but SOC only had a negative and positive relationship with clay and sand in Chinese red pine forest, respectively. Maybe the positive correlation was caused by this reason. Furthermore, except negative correlation in Chinese red pine forest between SOC, C: N ratio and MWD, all significant positive correlation in the two land use types between SOC, N, C: N ratio and MWD (Table 4). The reasons for this situation was likely duo to the increased cohesive interaction caused by the increased soil microbial activities induced by the polysaccharides (Tisdall et al., 1995). As this study found that soil microbial activities were exerted indirect restriction by the high C: N ratio in Chinese red pine forest.

The MWD of wet-sieved was only significant relevant with aggregate C: N ratio in Chinese red pine forest (Table 4). Moreover, the >1 mm aggregate C: N ratio had a significant negative correlation with MWD in Chinese red pine forest. But <1 mm aggregate C: N ratio had a positive significant correlation with MWD in natural restoration grassland. Table 1 also demonstrated that 1 mm were the main difference of the relationship between soil general properties and soil aggregate size fractions in the two land uses. Therefore, in this study area, 1 mm is a key aggregate size fraction that needs further research. In addition, the study showed that the relationship between MWD and SOC was a significant negative relevant in Chinese red pine forest (Table 4). Meanwhile, the relationship between GMD and N was also a significant negative relevant. This result is to some extent also proved that natural restoration grassland and Chinese red pine forest demand for carbon and nitrogen is just the opposite. All in all, the higher C content addition will further contribute the soil aggregate stability in grassland, and the higher N content will be more important in Chinese red pine forest, which the same with previous study (Bhattacharyya et al., 2012).

**Table 4**  
Pearson's correlations between aggregate chemical properties and soil aggregate stability index in five soil layers at soil depth of 0–100 cm (n = 25).

Land use type	Soil aggregate stability index	Aggregate size fractions						
		<0.25 mm	0.25–0.5 mm	0.5–1 mm	1–2 mm	2–5 mm	>5 mm	All
Grassland-SOC	GMD-D	−0.821**	−0.862**	−0.851**	−0.889**	−0.852**	−0.740**	−0.599*
	MWD-W	0.622**	0.606**	0.666**	0.454*	0.613**	0.575**	0.249*
	GMD-W	0.854**	0.842**	0.869**	0.701**	0.852**	0.812**	0.369*
	PAD	−0.895**	−0.886**	−0.915**	−0.743**	−0.897**	−0.865**	0.134*
Chinese red pine forest-SOC	GMD-D	−0.759**	−0.781**	−0.797**	−0.777**	−0.741**	−0.771**	
	MWD-W							−0.235*
	GMD-W	0.649**	0.636**	0.609**	0.603**	0.583**	0.568*	0.475*
	PAD	−0.877**	−0.861**	−0.862**	−0.853**	−0.818**	−0.801**	−0.271*
Grassland-N	GMD-D	−0.814**	−0.837**	−0.878**	−0.877**	−0.601*	−0.594*	−0.848*
	MWD-W	0.692**	0.661**	0.608**	0.686**	0.612**	0.556**	0.327*
	GMD-W	0.872**	0.871**	0.839**	0.881**	0.794**	0.713**	−0.599*
	PAD	−0.910**	−0.907**	−0.879**	−0.920**	−0.811**	−0.719**	−0.700**
Chinese red pine forest-N	GMD-D	−0.787**	−0.712**	−0.756**	−0.853**	−0.792**	−0.744**	−0.808**
	MWD-W							0.219**
	GMD-W	0.729**	0.671**	0.626**	0.660**	0.685**	0.758**	0.619**
	PAD	−0.935**	−0.886**	−0.859**	−0.893**	−0.905**	−0.927**	−0.742**
Grassland-C/N	MWD-W	0.657**	0.468**	0.414*				0.643*
	GMD-W	0.473**	0.438**	0.468**				0.560*
	PAD	−0.760*						
Chinese red pine forest-C/N	MWD-W				−0.634**	−0.511*	−0.613**	−0.677*
	GMD-W				−0.673**	−0.724*	−0.785**	−0.788**

Dry sieved (D), Wet sieved (W).

\* Correlation significant at the 0.05 level (2-tailed).

\*\* Correlation significant at the 0.01 level (2-tailed).

#### 4.4. Management implication in this area

Zhang et al. (2016) have found that the soil water storage decreased with the long-term natural vegetation restoration on the Loess Plateau, meanwhile, An et al. (2008) indicated that soil wet aggregate stability, mean aggregate diameter decreased with years following deforestation and concluded that soil erosion was primary process responsible for the degradation of measured soil physical, chemical, and microbiological properties. These results suggest the natural grassland can be restored to a forest even the lower soil aggregate stability and soil nutrient content. However, if there's no artificial management, Chinese red pine forest may be perished due to the unreasonable soil C: N ratio. Therefore, adjusting the ration of C to N is very necessary in artificial vegetation in the study area. However, this research only considered the soil information, so it is difficult to make a comprehensive view. Hence, nutrition addition, gap disturbances, soil microorganisms and plant information would be profitable for further research on the Loess Plateau.

#### 5. Conclusions

Overall, after ~15 years restoration from abandoned cropland, natural restoration grassland had higher SOC, TN, AN, MBN and MWD compared to planting Chinese red pine forest on the Loess Plateau, China. However, soil aggregate C and N under Chinese red pine forest was significantly greater than under natural restoration grassland. SOC positively and negatively correlated with MWD in natural restoration grassland and in Chinese red pine forest, respectively. Moreover, the differences of soil general properties and aggregate size fraction percentages between two land use types were found mainly in 2–5 mm, 1–2 mm, 0.25 mm and clay water-stable aggregate size fractions. The results indicated that the higher C content addition will further contribute the soil aggregate stability in natural restoration grassland, and the higher N content will be more important in Chinese red pine. Therefore, the results can provide some useful suggestions for land management on the Loess Plateau.

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