The distribution of soil microbial parameters based on aggregate fractions in successional grassland restoration ecosystems on the Loess Plateau

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Abstract

Soil microbial biomass and microbial metabolic quotients have been identified as the most effective indicators of a response to ecosystem development and disturbance. In order to determine the effects of grassland restoration programs, we analyzed the distribution of soil microbial parameters in different aggregate fractions at 1 yr. (1 year), 10 yr. (10 years) and 30 yr (30 years). Results show that C_{mic} (soil microbial biomass carbon) and P_{mic} (soil microbial biomass phosphorus) increased with grassland succession, the greatest values were concentrated at a micro-aggregate size. However, qCO_2 (soil microbial metabolic quotient) decreased as the years under restoration increased. qCO_2 was significantly correlated with SOC (soil organic carbon), TN (soil total nitrogen), P_{mic} (negatively) and C_{mic} (positively). Natural succession of grassland alters soil microbial properties positively, which plays a major role in aggregate formation. The time under vegetation rehabilitation impacts soil microbial parameters at different aggregate sizes, macro-aggregate size is the key factor in soil stability improvement in the process of natural restoration, especially for the aggregate size of 2–3 mm.

Keywords Vegetation restoration · Recovered chronosequence · Soil microbial parameters · Soil microbial metabolic · Soil aggregate size

1 Introduction

Soil microbes are known to play a key role in terrestrial ecosystem functions, such as carbon and nutrient cycling, plant growth stimulation, and degradation of organic matter [24]. Vegetation restoration is regarded as an effective strategy for controlling intensive soil erosion and its extensive effects [7]. Grassland is the most common terrestrial ecosystem on Earth with a crucial function of regulating climate and maintaining a balanced ecosystem [5]. The Loess Plateau of China covers 62.38×10^6 ha which includes 20.15×10^6 ha of grassland, with 91.2% of that area being occupied by natural grassland. But widespread stress practice, intensive cultivation, overgrazing

and large-scale monocultures have caused the area to acquiesce to soil erosion and a series of related eco-environment problems [11]. The Grain to Green program, a national ecological restoration program implemented in 1999, has made remarkable advances in vegetation recovery on the Loess Plateau [10]. Several countermeasures have been implemented including eco-environment rehabilitation using engineering and biological approaches [48]. Natural grassland protection and restoration is one of the important parts of a vegetation restoration program [23].

A growing body of ecological research on plant–microbe interactions in soil during vegetation restoration has recently been conducted. Positive and negative

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feedbacks between plants and soil microbes play central roles in early and later successional communities [38]. Soil structure, especially the spatial distribution of OM within the organic-mineral matrix of soil, exhibits control over microbial mediated decomposition processes in terrestrial ecosystems [29, 34]. Soil aggregates are structural units, where a group of primary soil particles cohere to each other more strongly than to other surrounding particles [4]. Research indicates that soil aggregates greatly impact the soil microbial biomass and mineral nutrients reserves [15, 46, 53]. Thus, the soil microbial biomass demonstrates a similarly positive relationship with soil structure, and microbial biomass shows significantly positive relationships with aggregate size and stability [12, 13, 36, 37]. When assessing the effects of disturbances on soil quality, Anderson and Domsch [3] proposed the ratio of soil basal respiration to microbial biomass (microbial metabolic quotient, specific respiration of the biomass or qCO_2), conceptually based on Odum's theory of ecosystem succession, is increasingly being used as an index of ecosystem development [30, 50]. Soil microbial biomass basal respiration and microbial metabolic quotient all respond readily to disturbance effects and provide an effective early warning for the deterioration of soil quality [8, 35, 49, 50, 57]. They have been used as indicators of change in soil organic matter that occur in response to land use alteration, tillage practices and soil pollution [9, 32].

We hypothesize that the years under grassland restoration effects soil structure formation which consequently effects the distribution of soil microbial biomass and microbial metabolic quotient. The objectives of this study are to explain (1) soil microbiological parameters in which grassland restoration year displayed more sensitive, in the middle of the succession or later period. and (2) which scope of soil aggregate sizes can reflect soil aggregate stability, What is the different between 1 yr and 30 yrs.

2 Materials and methods

2.1 Study site description

The study area was located in Chinese Loess Plateau, the south of Ningxia Province (106°25'–106°30' E, 35°59'–36°20' N). The region has a sub-arid climate characterized by seasonal rainfall with periodic local flooding and drought; the average annual temperature was 6°, and the average annual rainfall was 400–450 mm. The rainy season lasted from July to September and the rainfall in July accounting for 24% of the annual rainfall. Most of the land is at altitude of 1800–2040 m and is closely dissected by steep galleys [1]. Grassland soils from three restoration years (30 years, natural grassland; 10 years, natural

grassland; 1 year, abandoned grassland), were processed at the Yunwu Observatory for Vegetation Protection and Eco-environment.

2.2 Soil sampling collection

Undisturbed soil samples were collected from different restoration years in May 2016. For each restoration year, three sites in different locations were collected as replicates. An area of 50 m × 50 m was selected for each site and within this area three 10 m × 10 m plots were chosen for sampling. Soil profile was sampled by soil depth 0–20 cm. There were 5 replications for each site were randomly taken by 200 cm³ plastic boxes and composited to obtain a fresh sample. Samples were taken from each plot and mixed to a bulk sample of about 1 kg. After removing stones, roots and sieving at < 0.25 mm, 0.25–1 mm, 1–2 mm, 2–3 mm, 3–5 mm, > 5 mm, the sieved fresh soil samples were stored in ice boxes, and immediately transferred to the laboratory. Hence, we obtained 54 soil samples in total.

Care was taken during sample preparation to minimize moisture loss and samples were divided in two. One section was preserve in 4 °C for soil microbial measurements. The other was air dried for basic soil characteristics analysis. The water-holding capacity was determined by saturating each 100 cm³ turf sample with water and allowing it to drain at field capacity under cover for 48 h at ambient temperature; Duplicate soil cores were dried at 105 °C overnight to determine moisture content [1].

2.3 Sampling analysis

The soil samples were air dried and passed through a 2 mm sieve. The basic soil characteristics were analyzed through soil chemical and physical analyses [19]. Soil organic carbon was measured by wet digestion in a mixture of 5 ml of 0.136 mol L⁻¹ potassium dichromate and 5 ml of concentrated sulfuric acid. Soil total nitrogen was measured using Kjeldahl digestion. Alkali-ExTR-N was measured using a micro-diffusion method in which NH₃ was released from the soil sample by NaOH and then absorbed by boric acid. The ammonium borate product was titrated with 0.01 M HCl. Available phosphorus (Av-P) was extracted and measured in a buffered alkaline solution containing 0.5 M sodium bicarbonate. The extracts were quantified calorimetrically at 660 nm with a spectrophotometer (UV2300, Hitachi, Tokyo, Japan) [54]. The readily available potassium was extracted from the soil with 1 mol L⁻¹ NH₄OAc and was measured using flame photometry. The soil pH was measured in water (1/2.5 w/v), and the moisture content was assessed by drying the samples in an oven at 105° overnight.

Soil basal respiration was estimated through CO₂ evolution at 25 °C in samples incubated for 10 days [21]. Measurements were made in the laboratory at 50% of field water holding capacity (WHC). CO₂ respired was trapped in NaOH, and the residual NaOH was titrated with HCl [1].

The soil microbial biomass C, N and P levels were determined using the fumigation-extraction method. A 15 g sample of oven-dried, field-moist-equivalent soil (< 2 mm) was fumigated with chloroform for 24 h with the chloroform being removed by repeated evacuation. The soil was reinoculated with a small amount of unfumigated soil and incubated at a constant temperature (usually 22 or 25) for 10 days at field capacity or at 50% of its water-holding capacity (approximately – 0.01 MPa). An additional, unfumigated soil sample was used as a control [20, 21]. The soil microbial biomass carbon level was determined immediately using a TOC analyzer or the sample was preserved at – 18 (Phoenix 8000, Tekmar–Dohrmann, Mason, OH, U.S.A.). A k_c value of 0.4 was chosen to calculate the total soil microbial biomass C [51]. To determine soil microbial biomass N, fumigated and unfumigated samples were extracted via potassium persulfate oxidation and then measured using ultraviolet spectroscopy. The extract liquor was digested with alkaline auto-oxidation using 0.15 mol L^{–1} NaOH and 30 g L^{–1} K₂S₂O₈ with a 1:1 v/v mixture of oxidant and soil at 120–124 for 30 min [54]. The concentration of N was determined colorimetrically using a spectrophotometer (UV2300, Hitachi, Tokyo, Japan) at 220 and 275 nm. A k_N value of 0.54 was chosen to calculate the total soil microbial biomass N [47]. The soil microbial P level was determined calorimetrically with a spectrophotometer. 2.5 g of fumigated and unfumigated soil was placed into a 150-ml flask containing 50 ml of 0.5 mol L^{–1} NaHCO₃ solution and 2 g of P-free active charcoal. 5 ml of Mo-Sb spectrophotometry solution was added for color development. After 30 min, the color was determined with a spectrophotometer (UV2300, Hitachi, Tokyo, Japan) at 700 nm. The microbial P was calculated using a k_p factor of 0.40 [2, 14].

2.4 Statistical analysis

Variety statistical analyses were conducted using SPSS 18.0 to identify the effects of the successional grassland restoration years on soil microbiological parameters. One-way analysis of variance (ANOVA) with restoration years and soil aggregate sizes as the main factors were employed to test the significance of the mean differences. The level set for a significant difference was $p < 0.05$. A univariate procedure was used to examine the normality of the distribution of the observed data before conducting the ANOVA analysis. When the ANOVA test indicated a significant effect at $p < 0.05$, the means of each soil variable for each classification factor were assessed using the multiple-mean comparison that was assessed using the Benjamini False Discovery Ratio method. In order to explore the relationship between soil microbial biomass and soil physiochemical properties, we used canonical correlation analysis which is more effective analysis than the simple correlation analysis (Table 1).

3 Results

3.1 Basic soil characteristics

General soil characteristics for the various restoration years are shown in Table 2. As grassland recovery years increased, maximum field capacity (MFC), porosity (Por.), soil organic carbon (SOC), total nitrogen (TN), rapid available phosphorus (Ap-P), invertase (Inv), Alkal-phosphatase (Alk.-P), soil microbial biomass carbon (C_{mic}) and phosphorus (P_{mic}) also increased. However, restoration years also resulted in a decrease in bulk density (BD). The highest concentrations of soil microbial biomass nitrogen (N_{mic}), urease (Ure) and rapid available potassium (Ap-K) were found in 10 year grassland. The distribution of aggregate size class percentage varied among the grassland restoration sites (Fig. 1). The percentage of < 0.25 and > 5 mm aggregate sizes in 30 year natural

Table 1 Sampling site characteristics

Successional years	Dominant species	Accompanying species	Elevation (m)	Slope gradient (°)
30	<i>Thymus mongolicus</i> <i>Stipa bungana</i>	<i>Stipa grandiss</i> <i>Artemisia sacrorum</i> . <i>Artemisia scoparia</i>	1930	12
10	<i>Leymus secalinus</i> <i>Thymus mongolicus</i>	<i>Artemisia scoparia</i> <i>Potentilla bifurca</i> <i>Stipa bungana</i>	1908	11
1	<i>Artemisia scoparia</i> <i>Potentilla bifurca</i>	<i>Thymus mongolicus</i> <i>Heteropappus altaicus</i> <i>Thymus mongolicus</i> <i>Stipa bungana</i>	1940	10

Table 2 Basic soil characteristics

Soil characteristics	Successional years		
	30 years	10 years	1 year
BD	0.91	1.13	1.28
MFC	37.08	31.83	27.34
Por.	54.85	54.80	50.36
SOC	27.85	18.15	9.50
TN	3.10	1.88	1.17
Ap-P	3.56	3.37	2.71
Ap-K	166.24	241.69	153.04
NH ₄ ⁺ -N	14.08	4.21	4.53
NO ₃ ⁻ -N	16.62	6.40	15.06
C:N	8.87	8.26	8.26
Inv.	20.40	14.77	7.83
Alk.-P	12.43	9.99	5.95
Ure.	2.62	4.13	3.64
C _{mic}	1065.49	683.72	358.96
N _{mic}	25.78	41.41	17.23
P _{mic}	19.99	15.74	2.56

BD: soil bulk density (g cm³); MFC: maximum field capacity (%); Por.: porosity (%); SOC: soil organic carbon (g kg⁻¹); TN: total nitrogen (g kg⁻¹); Ap-P: rapid available phosphorus (mg kg⁻¹); Ap-K: rapid available potassium (mg kg⁻¹); NH₄⁺-N: soil ammonium nitrogen (mg kg⁻¹); NO₃⁻-N: soil nitrate nitrogen (mg kg⁻¹); Inv.: invertase (mg g⁻¹); Alk.-P: Alkal-phosphatase (mg g⁻¹); Ure.: urease (mg g⁻¹); C_{mic}: soil microbial biomass (mg kg⁻¹); N_{mic}: soil microbial nitrogen (mg kg⁻¹); P_{mic}: soil microbial phosphorus (mg kg⁻¹)

Fig. 1 Effect of restoration years on the distributions of aggregate sizes' percentage

grassland was lower than in 10 year. Lastly, soil aggregate composition in 30 year grassland was mainly concentrated between 0.25 and 5 mm.

3.2 Distribution of SOC and TN in various aggregate sizes

The concentration of SOC and TN associated with soil grain size increased with the number of years of grassland

succession (Fig. 2a, c). For 10- and 30-year natural grassland, the percentage of SOC in 1–2, 2–3 and 3–5 mm was higher than for other aggregate sizes. In 1 year abandoned grassland, the macro-aggregate size (> 5 mm) showed the greatest SOC with rapid enhancement between 3–5 and 5 mm (Fig. 2b). The trend in distribution of TN among the various aggregate classes in 10- and 30-year natural grassland was similar (i.e., stable between 15.79 and 17.64). However, significant changes occurred in 1 year-abandoned grassland (Fig. 2d). The percentage of TN (11.26) was lowest for micro-aggregates (<0.25 mm), highest in 0.25–1 mm (19.22), and remained at a lower yet steady level for 10- and 30-year (17.02–18.51).

3.3 The distribution of soil microbial parameters at different aggregate sizes

3.3.1 Soil microbial biomass

The concentrations of C_{mic} and P_{mic} at different grain sizes increases with length of grassland succession (Fig. 3a, e). The situation with N_{mic} is different where it had the highest value in a 10 year natural grassland (Fig. 3c).

Under a 30 year natural grassland, the percentage of C_{mic} (18.59) was greatest for a micro-aggregate size of <0.25 mm, as aggregate size increased, percentages dropped to a stable range between 15.68 and 16.56. At 10 years, a grain size of 1–2 mm showed the greatest percentage of C_{mic} (18.4), which was a rapid increase from <0.25. The percentage of C_{mic} for other particle sizes remained at a stable level between 16.72 and 17.61 (Fig. 3b). The curve of C_{mic} versus aggregate size for a recently abandoned grassland fluctuated greatly. There was a significant decrease in percentage of C_{mic} in 2–3 mm versus 1–2, 3–5 and > 5 mm. In a 30-year natural grassland, the percentage of N_{mic} remained stable between 14.99 and 18.48 for the various aggregate sizes. As soil aggregate size increased, the distribution of N_{mic} in 1- and 10-year grassland showed a rising tendency and declining trend, respectively (Fig. 3d). The percentage of P_{mic} in different aggregate sizes for 10- and 30-year natural grassland remained stable. Whereas, for the highest percentage occurring in aggregate sizes of 2–3 mm, the 1-year curve showed a “normal distribution” which was significantly enhanced from 0.25–1 mm and rapidly declined at 2–3 mm (Fig. 3f).

3.4 Soil microbial basal respiration and metabolic quotient

Soil microbial basal respiration (SBR) was enhanced as the successional years increased (Fig. 4a). For a grassland abandoned for 1 year, the percentage of SBR in <0.25

Fig. 2 Effect of restoration years on SOC and TN for different aggregate sizes. **a** The distribution of SOC in different vegetation restoration years; **b** The percent distribution of SOC in soil aggregate size; **c** The distribution of TN in different vegetation restoration years; **d** The percent distribution of TN in soil aggregate size. Different

capital letters within columns indicate statistically significant differences among the different restoration years, different lowercase letters within columns indicate statistically significant differences among the different soil aggregate size ($p < 0.05$)

and 0.25–1 mm was lower than in other aggregate sizes. SBR increased with aggregate size, the highest occurring between 3 and 5 mm. The curve of SBR for a 30-year natural grassland for different aggregate size classes fluctuated, it was the highest in 3–5 mm and lowest in 2–3 mm. However, the percentage at 10 years increased with soil aggregate size (Fig. 4b). Soil microbial metabolic quotient ($q\text{CO}_2$) among the aggregate size classes had the opposite reaction as restoration years increased (Fig. 4c). In a grassland abandoned for 10 years, the percentage of $q\text{CO}_2$ in aggregate size classes of 0.25–1, 1–2 and 2–3 mm, was lower than in the micro-aggregate (<0.25 mm) and macro-aggregate (3–5 mm and >5 mm) classes (Fig. 4d).

3.5 The ratios of C/N, $C_{\text{mic}}/N_{\text{mic}}$ and $C_{\text{mic}}/P_{\text{mic}}$

The ratios of C/N, $C_{\text{mic}}/N_{\text{mic}}$ and $C_{\text{mic}}/P_{\text{mic}}$ for the different aggregate sizes in the three successional periods were

shown in Table 3. The greatest ratios of C/N, $C_{\text{mic}}/N_{\text{mic}}$ and $C_{\text{mic}}/P_{\text{mic}}$ were exhibited in the 10 year, 30 year and 1 year grasslands, respectively.

For the 10- and 30-year natural successional grasslands, the ratios of C/N in aggregate size of 1–2, 2–3 and 3–5 mm were higher than the others. The ratios of $C_{\text{mic}}/P_{\text{mic}}$ in grassland under succession for 30 years was greater in <0.25, 0.25–1 and >5 mm and were between 43.11 and 46.25 in grassland under succession for 10 years, expect <0.25 mm which had the lowest value. For a grassland under restoration for one year, the ratios show a V shaped distribution among the soil aggregate sizes, with the lowest ratio occurring in 2–3 mm. The ratio of $C_{\text{mic}}/N_{\text{mic}}$ in grassland that was in a natural state for 30 years was maintained at a stable level (37.03–42.02), but, at 10 years, the ratio increased as soil aggregate size increased. For a grassland abandoned for 1 year, the ratio was greatest for <0.25 mm and lowest for >5 mm, the other ratios were between 9.32 and 25.96.

Fig. 3 Effect of restoration years on C_{mic} , N_{mic} and P_{mic} in different grassland restoration years; **a** The distribution of C_{mic} in different grassland restoration years; **b** The percentage distribution of C_{mic} in soil aggregate size; **c** The distribution of N_{mic} in different grassland restoration years; **d** The percentage distribution of N_{mic} in soil aggregate size; **e** The distribution of P_{mic} in different grassland restoration

years; **f** The percentage distribution of P_{mic} in soil aggregate sizes. Different capital letters within columns indicate statistically significant differences among the different restoration years, different lowercase letters within columns indicate statistically significant differences among the different soil aggregate size ($p < 0.05$)

Fig. 4 Effect of restoration years on SBR and $q\text{CO}_2$ in different aggregate sizes. **a** The distribution of SBR for different grassland restoration years; **b** The percent distribution of SBR in soil aggregate sizes; **c** The distribution of $q\text{CO}_2$ for different grassland restoration years; **d** The percent distribution of $q\text{CO}_2$ in soil aggregate

sizes. Different capital letters within columns indicate statistically significant differences among the different restoration years, different lowercase letters within columns indicate statistically significant differences among the different soil aggregate size ($p < 0.05$)

3.6 The relationship of soil microbial parameters and chemical characteristics

Significant correlations were found between soil microbial properties and chemical characteristics. As Table 4 shows, a correlation exists among the soil microbial properties that were significantly correlated with SOC, TN and Ap-P. N_{mic} did not relate to SBR and $q\text{CO}_2$, and only correlated with NO_3^- -N. C_{mic} and P_{mic} were significantly correlated with SBR, $q\text{CO}_2$ and $C_{\text{mic}}/N_{\text{mic}}$ ($p < 0.01$). The ratio of $C_{\text{mic}}/P_{\text{mic}}$ was negatively correlated with C_{mic} ($R = -0.638$, $p < 0.01$), N_{mic} ($R = -0.507$, $p < 0.05$), P_{mic} ($R = -0.833$, $p < 0.01$) and SBR ($R = -0.727$, $p < 0.01$), and positively correlated with $q\text{CO}_2$ ($R = 0.635$, $p < 0.01$).

Table 5 shows that soil microbial metabolic quotient ($q\text{CO}_2$) was positively related to C_{mic} ($R = 0.954$) and negatively correlated to P_{mic} ($R = -0.901$), the correlation ratios were significant ($p < 0.01$). The correlation between C_{mic} and $q\text{CO}_2$, P_{mic} and $q\text{CO}_2$ in different aggregate sizes is shown in Figs. 5 and 6. The correlation coefficients for C_{mic}

and $q\text{CO}_2$, P_{mic} and $q\text{CO}_2$ in <0.25, 0.25–1, 1–2 and >5 mm were higher (0.9177–0.9804, 0.8288–0.989) than in 2–3 (0.7427, 0.7679) and 3–5 mm (Fig. 5d, f).

4 Discussion

Soil microorganisms are potential indicators involved in several biological processes [56] and can be used to evaluate the success of revegetation in disturbed soil ecosystems [1]. Simultaneously, the formation and stability of soil aggregates are affected by many factors, such as plant roots, microorganisms, and soil organic matter content, and their interactions [40]. Following the long-term grassland succession, it is still unclear how vegetation restoration affects the soil aggregate distribution and soil microbial characteristics in aggregates of different size classes, as well as the potential interaction between soil microorganisms and soil aggregate size.

Table 3 The ratios of C/N, C_{mic}/N_{mic} and C_{mic}/P_{mic} for different aggregate sizes

Successional years	Size classes (mm)	C/N	C_{mic}/P_{mic}	C_{mic}/N_{mic}
30	< 0.25	8.42	67.38	44.92
	0.25–1	8.67	56.05	37.03
	1–2	9.51	49.89	44.57
	2–3	9.35	52.98	37.37
	3–5	9.32	41.93	43.24
	> 5	8.52	56.01	42.02
Mean		8.97	54.04	41.53
10	< 0.25	8.95	36.09	10.30
	0.25–1	8.67	43.11	14.08
	1–2	10.12	44.13	15.91
	2–3	10.08	45.25	17.41
	3–5	9.81	46.87	21.02
	> 5	10.23	44.53	27.04
Mean		9.64	43.33	17.62
1	< 0.25	10.40	206.01	46.49
	0.25–1	6.85	218.87	20.67
	1–2	7.56	119.22	25.96
	2–3	8.29	64.21	19.32
	3–5	7.19	170.75	20.10
	> 5	9.28	290.95	13.58

Soil microbial biomass, soil basal respiration and metabolic quotient are commonly used as “bio-markers” to assess soil quality [25], and have been previously considered to be sensitive parameters of evaluating natural and

degraded systems [6, 55]. In this study, the results showed that soil microbial biomass C and P were enhanced in the vegetation restoration, and this enhancement was positively correlated with soil micro-aggregate size. A widely accepted theory of soil aggregation formation is the phase theory proposed by Tisdall and Oades [44], which stated that soil micro-aggregates are generated from soil mineral particles through binding of multivalent cations and soil organic matter, and then the micro-aggregates are held together by plant roots and fungal hyphae to gradually form larger aggregates. Although the variation in different soil aggregate proportions was not correlated with the amount of soil microbial indicators, the C_{mic} content in micro-aggregate size class (< 0.25, 0.25–1, 1–2 mm) was linearly correlated with restoration years increased.

Such interactions between vegetation and soil microbes are reflected by the fact that changes in microbial composition, activity, and biomass largely resulted from the higher litter quality of vegetation associated with great diversity [58]. In the present study, SOC, TN and Ap-P (chemical characteristics); Inv., Alk-P (soil enzyme activities); C_{mic} and P_{mic} (soil microbial biomass) increased with an increase in restoration years. This finding is similar with a previous study conducted by Yan et al. [55] who reported soil microbial community composition significantly changed with vegetation succession. Gupta and Germida [13] proposed the microbial biomass plays an important role in the formation of macro-aggregates and is the primary resource of labile organic carbon and nutrients. Relative to the macro-aggregates (> 0.25 mm) contained lower organic carbon, microbial biomass, fungal

Table 4 Correlation coefficients between basic characteristics and soil microbial parameters

	SOC	Total N	NO_3-N	NH_4-N	Ap-K	Ap-P	C/N
C_{mic}	0.965**	0.964**	0.155	−0.269	0.087	0.574*	0.259
N_{mic}	0.268	0.206	−0.704**	−0.106	0.046	0.035	0.312
P_{mic}	0.933**	0.883**	−0.106	−0.208	0.406	0.677**	0.422
SBR	0.910**	0.882**	0.046	−0.098	0.207	0.672**	0.326
qCO_2	−0.932**	−0.906**	0.035	0.395	−0.267	−0.473**	−0.463

*Significant at the 0.05 level; **Significant at the 0.01 level

Table 5 Correlation coefficients for soil microbial biomass and respiration

	C_{mic}	N_{mic}	P_{mic}	SBR	qCO_2	C_{mic}/N_{mic}	C_{mic}/P_{mic}
C_{mic}	1						
N_{mic}	0.250	1					
P_{mic}	0.903**	0.486**	1				
SBR	0.917**	0.305	0.933**	1			
qCO_2	0.954**	−0.414	−0.901**	0.841**	1		
C_{mic}/N_{mic}	0.593**	0.548**	0.369	0.472*	−0.463	1	
C_{mic}/P_{mic}	−0.638**	−0.507*	−0.833**	−0.727**	0.635**	0.142	1

*Significant at the 0.05 level; **Significant at the 0.01 level

Fig. 5 The relationship between C_{mic} and qCO_2 among different aggregate sizes

Fig. 6 The relationship between P_{mic} and qCO_2 among different aggregate sizes

biomass, and respiratory activity, with a slow turnover rate of organic carbon.

Natural grassland flourished when grazing was prohibited. The vegetative species and composition change as the vegetation restoration years increase, which impacts the soil microbial community in a direct way. An extensive root system provides the most improvement in soil structure by binding macro-aggregates to fine roots and VAM fungal hyphae and by binding micro-aggregates with adhesive bacterial metabolic products [26, 45, 52]. The natural grassland with 30 years restoration has a species-rich plant community that protects soil from physical disturbance [33], and higher soil organic matter supports

an abundant carbon source for the growth of microorganisms and metabolism [41, 42], which results in the highest amounts of C_{mic} and P_{mic} . Simultaneously, the percentage of C_{mic} at different aggregate sizes was stable [43].

Soil's basal respiration indicates the general activity or energy expended by its own microbial pool. The ratio of soil basal respiration to microbial biomass was the most sensitive indicator of quantitative and qualitative changes in microbial communities caused by various management systems [20]. Soil respiration relies on a suite of complex processes contributing to CO_2 efflux from soil surface, mainly from plant roots and micro-organisms [22]. Many

studies showed the changes in soil respiration and soil microbiological properties with succession [27, 28, 39].

In this study, soil basal respiration was enhanced as natural restoration years increased with the highest concentrations found at the macro-aggregate level. The microbial carbon utilization is denoted by metabolic CO_2 quotient. The findings of Insam and Haselwandtere [17] on two 50-year-old soil chronosequences on reclamation sites are similar. However, soil microbial metabolic quotient ($q\text{CO}_2$) declined as vegetation restoration years increased. In addition, some authors reported that systems having high organic matter inputs and organic compounds readily available in the soil tended to have greater microbial biomass content and activity since these are the preferred energy sources of microorganisms. The $q\text{CO}_2$ considers both basal respiration and C_{mic} and has been mentioned as a superior indicator for microbial metabolic efficiency compared with basal respiration alone, particularly when differentiating soil biota responses to soil management approaches [16].

The percentage of $q\text{CO}_2$ in micro-aggregate sizes was lower than in macro-aggregate sizes, the distributions of SOC and TN in different aggregate sizes was similar. Because $q\text{CO}_2$ was negatively correlated with SOC and total N, the decrease in $q\text{CO}_2$ with time for the vegetated areas at different aggregate sizes resulted in competition for the available carbon source, which seemed to favor aggregate classes that needed the least amount of energy for maintenance and growth [17]. In the current study, relationships existed between $q\text{CO}_2$ and SOC, TN, Ap-P, C_{mic} , P_{mic} and SBR. Especially for C_{mic} and P_{mic} , the ratios in 2–3 mm were lowest (0.7472 and 0.7679, respectively). This is not only due to decreases in microbial biomass and increases in respiration, but also to the diversion by soil microorganisms of growth energy toward maintenance [31]. A higher $q\text{CO}_2$ and a lower microbial biomass may both occur late in ecosystem succession, and the relationship between the two reflects their dual response to underlying stress [50]. The result demonstrate that 2–3 mm is the relative stable aggregate size class in ecosystem succession.

5 Conclusion

Loess Plateau ecosystems are suffering serious environmental problems including natural grassland degradation due to soil and water erosion. This study shows that grassland has a positive effect on soil status as indicated by an increase in the concentration of soil nutrients and microbial biomass. As grassland restoration years increased, soil microbial biomass carbon, phosphorus and basal respiration increased. Soil microbial metabolic quotient ($q\text{CO}_2$)

an alternative measure that changes when microbial biomass responses to disturbances, is significantly correlated to C_{mic} and P_{mic} , the lowest ratios illustrated that 2–3 mm was the stablest aggregate size in the process of ecological restoration.

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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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