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Effects of Legume–Grass Ratio on C and Nutrients of Root and Soil in Common Vetch–Oat Mixture under Fertilization

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Abstract: Legume–grass mixture can greatly improve soil fertility to support the sustainable productivity. Root litter is an important source of soil organic matter, but its link with soil nutritional status in forage mixtures is not clear. This study was aimed to uncover whether the relationship of carbon (C) and nutrients between root and soil would change with mixing ratio. Changes in organic C, nitrogen (N), and phosphorus (P) of root and soil were studied in a 2-year experiment with sole common vetch (*Vicia sativa*), sole oat (*Avena sativa*), and their mixtures of different mixing ratios under N, P, and N + P fertilization. Root C, N, and P concentrations decreased with decreasing proportion of common vetch in the grasslands. Nitrogen fertilization significantly improved root N concentration (by 4.5–10.1%), while P fertilization decreased root N concentration (by 10.1–18.4%). The effect of mixing ratio on soil C and nutrients was stimulated by fertilization, although soil C, N, and P contents barely changed with mixing ratio. Mixing and fertilization significantly affected C, N, and P stoichiometric ratios of root and soil (besides soil C:N). Soil C, N, and P contents were strongly positively correlated with root C concentration. The results indicated that increasing legume proportion in the mixture may improve root C and nutrients, which can be stimulated by fertilization. Root quality is closely correlated to soil nutritional status in the mixture. This study further reveals the mechanism how the root is potentially involved in affecting soil fertility and provides a scientific basis on the extensive use of common vetch-oat mixture in the Loess Plateau of China.

Keywords: fertilization; legume–grass mixture; intercropping; mixing ratio; nutritional status; root–soil interaction

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1. Introduction

The root plays an important role in energy flow and material circulation of the terrestrial ecosystem. It absorbs nutrients and water from soils, consumes photosynthetic products through respiration and turnover, and returns organic matter into soils through decomposition [1]. The decomposition of root litter and the release of carbon (C) and nutrients into soils affect nutrient cycling and soil fertility [1]. The stoichiometry of C, nitrogen (N) and phosphorus (P) of root and/or soil has been explored in different terrestrial ecosystems, such as natural grassland and forest [2,3], but the relationship of C and nutrients between root and soil is far from known in diverse vegetation systems, for instance, the cultivated grassland.

As an efficient pattern of cultivated grassland, the mixture of multiple forages is dominant in changing soil nutrient characteristics. Compared with the monoculture, soil C and nutrient storages are stronger in the mixtures [4,5], which is partly attributed to stronger ability to return organic matter [6]. Plant species and the proportion of mixed components affect soil organic matter [7]. In temperate grasslands, the increase in legume proportion significantly enhances soil C storage, while legume proportion higher than 50–75% restricts soil N storage [8] due to the influence of intraspecific competition and interspecific interaction between legume and non-legume, which decreased N transfer and probably inhibited biological N_2 fixation (BNF) [9], while increasing the utilization of soil N by plant and restraining soil N accumulation. The unique BNF can not only provide an extra N source for the legume, but also help enhance the availability of soil nutrients [10]. Greater N concentration and faster decomposition in legume–grass mixtures result in greater litter N release than the monocultures [11]. In addition, increasing N fertilization leads to increased litter decomposition rate [12,13]. Root litter shows advantages to return organic matter in the mixtures, playing an important role in improving soil fertility and sustaining the productivity.

Root and soil share close coupling in nutrients. Due to the heterogeneity of soil available resources, root nutrient concentrations usually vary spatially [14]. The release of root exudates and the decomposition of root litter are important sources of soil organic matter [15]. Intercrops (including mixing crops) have greater root productivity, which can greatly help increase soil quality and C sequestration [16]. In addition, root chemistry seems to be one of the main factors controlling root litter decomposition [17]. The decomposition rate is significantly negatively correlated to root C:N in natural and semi-natural ecosystems [18]. Therefore, it is very important to deeply understand the relationship of C and nutrients between root and soil; however, few studies have been carried out to uncover how mixing ratio affects root C and nutrients and consequently affects soil nutritional status.

In the Loess Plateau of China where the soil is infertile due to serious soil erosion and inappropriate farming, fertilization has long been a common management practice to increase the availability of soil nutrients [19]. However, unsuitable fertilizer application has led to serious issues such as high economic and labor inputs, environmental pollution etc. In contrast, inclusion of legume and grass into arable lands is an efficient measure to restore soil fertility in this area [20]. Common vetch (*Vicia sativa*)–oat (*Avena sativa*) mixture has shown great advantages in maintaining productivity and improving resource utilization [21,22]. Further clarifying relationships of C and nutrients between root and soil in common vetch–oat mixtures help some in the extensive use of such mixture in this area.

In this study, we hypothesized that the effect of fertilization on root and soil C and nutrients, and C:N:P would change with mixing ratio in the mixtures. The objectives were: (1) to find out how C and nutrients of root and soil changed with mixing ratios and fertilization rates; (2) and to uncover the relationship between root and soil nutritional status.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted in Qingyang Loess Plateau Pastoral Agriculture Station (107°52′ E, 35°39′ N, altitude 1297 m asl) of Lanzhou University. It is a typical semi-arid rainfed agricultural area with continental monsoon climate. In 2019 and 2020, the annual precipitation was 668 and 580 mm (Figure 1), respectively, greater than the long-term average of 559 mm (1970–2018). More than 70% of the annual total falls during July to September. The soil is locally Heilu soil, an Entisol in the classification of the Food and Agriculture Organization of the United Nations, which is a silty loam containing 70% silt, 23% clay, and 7% sand, representing the major cropping soil of this area.

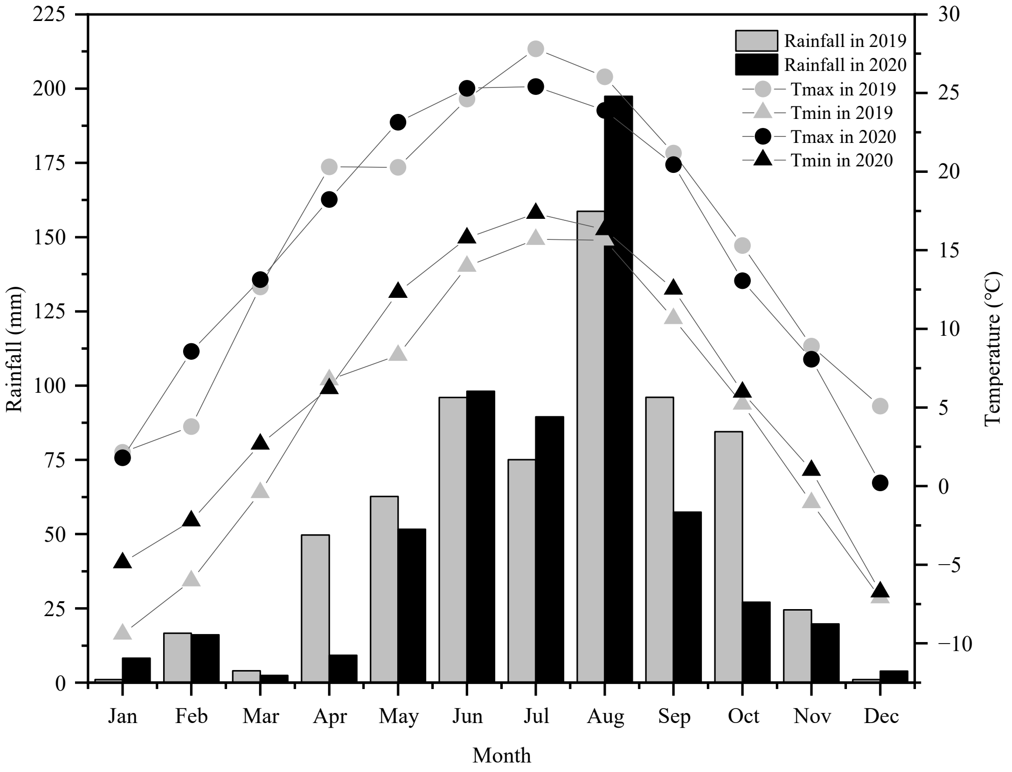


Figure 1. The monthly rainfall, maximum (Tmax) and minimum air temperatures (Tmin) in 2019 and 2020.

Before oat (*A. sativa* cv. Galileo) and common vetch (*V. sativa* cv. Lanjian 2), sorghum (Sorghum bicolor) in 2018 or corn (Zea mays) in 2019 was sown in the experimental fields as preceding crop. The basic feature in 0–30 soil layer was shown in Table 1.

Table 1. Basic soil feature at the experimental site.

Year	Layer (cm)	pH	Organic C (g/kg)	Nitrate N (mg/kg)	Ammonium N (mg/kg)	Total N (g/kg)	Available P (mg/kg)	Total P (g/kg)
2019	0–10	8.22	9.73 ± 0.14	14.81 ± 3.83	1.12 ± 0.07	1.01 ± 0.01	18.85 ± 2.14	0.51 ± 0.02
	10–20	8.20	8.88 ± 0.08	13.03 ± 2.33	1.03 ± 0.17	0.97 ± 0.01	22.13 ± 3.17	0.53 ± 0.02
	20–30	8.11	9.32 ± 0.13	13.99 ± 0.79	0.94 ± 0.07	0.91 ± 0.01	17.28 ± 3.93	0.59 ± 0.03
2020	0–10	8.12	5.02 ± 0.19	11.13 ± 0.18	0.57 ± 0.03	0.61 ± 0.03	10.97 ± 0.26	0.39 ± 0.01
	10–20	8.11	3.82 ± 0.27	12.55 ± 0.44	0.77 ± 0.05	0.63 ± 0.01	11.10 ± 0.38	0.36 ± 0.02
	20–30	8.14	4.98 ± 0.27	13.95 ± 0.63	0.62 ± 0.05	0.56 ± 0.01	6.30 ± 0.73	0.36 ± 0.02

Values are presented as mean ± S.E. (n = 3).

2.2. Experimental Design

The field treatments were arranged in a randomized complete block design with mixing and fertilization as two factors in 2019 and 2020, (Table 2). The ratio of common vetch and oat in the grasslands were set as 1:0 (V), 2:1 (VA21), 1:2 (VA12), and 0:1 (A). The sowing rates were common vetch 75 kg/ha (V), common vetch 50 kg/ha and oat 30 kg/ha (VA21), common vetch 25 kg/ha and oat 60 kg/ha (VA12), and oat 90 kg/ha (A), respectively. The seeds were sown in rows at 1–2 cm depth on 26 April 2019 in the N test and on 20 June 2020 in the N + P test. Fertilizers were applied prior to planting with N fertilization in the form of urea (N $\geq$ 46%) and/or P fertilization in the form of calcium super phosphate ($P_2O_5 \geq 16.0\%$). For the N test in 2019, four N fertilization rates were set including 0 (N0), 50 (N50), 100 (N100), and 150 kg/ha (N150) with 60 kg P_2O_5/ha as base fertilizer. For the N + P test in 2020, six fertilization rates were set including 0 (CK), 60 kg P_2O_5/ha (P1), 120 kg P_2O_5/ha (P2), 100 kg N/ha (N), 100 kg N/ha

+ 60 kg P_2O_5/ha (NP1), and 100 kg N/ha + 120 kg P_2O_5/ha (NP2). No other fertilization was carried out during the experimental period. Each treatment had three replicate plots and all plots were 4 m × 3 m with 30 cm alley between plots. During the experimental duration, there was no irrigation and it was completely rainfed. Constant weed, pest, and disease controls were carried out as locally recommended. Both forages were harvested at the flowering stage of common vetch (26 June 2019, or 1 September 2020). In 2019, the duration of growth was 64 d and in 2020, it was 74 d due to contrasting rainfall and temperature, both of which led to a delayed sowing and slow growth.

Table 2. Experimental design for fertilization and mixing treatments in 2019 and 2020.

Treatment	N Test in 2019	N + P Test in 2020
Fertilization	No N fertilization (N0) 50 kg N/ha (N50) 100 kg N/ha (N100) 150 kg N/ha (N150)	No N and P fertilization (CK) 60 kg P_2O_5/ha (P1) 120 kg P_2O_5/ha (P2) 100 kg N/ha (N) 100 kg N/ha + 60 kg P_2O_5/ha (NP1) 100 kg N/ha + 120 kg P_2O_5/ha (NP2)
Mixing	Mixing ratios of common vetch and oat were 1:0 (V, 100% common vetch), 2:1 (VA21, 62.5% common vetch + 37.5% oat), 1:2 (VA12, 29.4% common vetch + 70.6% oat), and 0:1 (A, 100% oat). Sowing rates were common vetch 75 kg/ha (V), common vetch 50 kg/ha and oat 30 kg/ha (VA21), common vetch 25 kg/ha and oat 60 kg/ha (VA12), and oat 90 kg/ha (A), respectively.	

2.3. Sampling, Measurement and Calculation

Immediately after harvesting (the flowering stage of common vetch) of both forages, root and soil samples were taken for further measurements. In each plot, a root drill (inner diameter 9 cm) was used to take soil cores at 0–10, 10–20, and 20–30 cm depth in between and within rows. Two soil cores from the same layer in each plot were combined into a single composite sample. Roots in the soil were collected with a mesh bag of 0.25 mm aperture, which was washed with tap water. According to the shape, color, and flexibility of the root, vital roots [23] were selected and then dried to constant weight at 65 °C to obtain root dry weight. The dried root sample was ground into fine powder to pass a 1.0 m sieve for further measurements of root C and nutrients. Soil sample was randomly taken using a soil drill (inner diameter 5 cm), referring to root sampling method, and air-dried naturally in a cool place. The dried soil sample was sieved through 0.25 mm sieve for further measurements of soil C and nutrients.

Organic C was measured using the H_2SO_4-$K_2Cr_2O_7$ oxidation and titration method. Total N was measured using the automatic Kjeldahl method with a Kjeldahl auto-analyzer (Kjeltech 8400, Foss, Denmark). Total P was determined using molybdenum antimony colorimetric method with a spectro-photometer (UV-2102 PCS, Metash, Shanghai, China). Root C (C_R), N (N_R), and P (P_R) concentrations were expressed on a dry weight basis. Soil C (C_S), N (N_S), and P (P_S) content were calculated on a mass basis and expressed as soil nutrient density (*SND*).

$$SND\left(\mathrm{mg/cm^2}\right) = \sum_{i=1}^{n} P_i \times C_i \times T_i$$

where i refers to soil layer; P_i refers to soil bulk density (g/cm^3) of i soil layer; C_i refers to C or nutrient content (mg/g) in i soil layer; T_i refers to the thickness (cm) of i soil layer; and n refers to the total number of soil layers.

2.4. Statistical Analysis

The analysis was performed using SPSS 21.0. Before analysis, variables (root C, N, P concentrations, soil C, N, P contents, and stoichiometric ratios) were checked for normality of distribution and homogeneity using the Shapiro–Wilk test. Two-way ANOVA was used to analyze the effects of fertilization and mixing treatments on the variables. The differences of the variable among mixing ratios and fertilization rates were examined using Duncan's test at a significance level of $p < 0.05$. The correlations between soil and root were determined using the "correlation plot" package in Origin Pro 2021.

3. Results

3.1. Root C, N, P Concentrations and Stoichiometric Ratios

In 2019, C_R, N_R, and P_R concentrations were significantly affected by mixing and its interaction with fertilization (Table 3). The C_R concentration tended to increase with the proportion of common vetch and was significantly greater in common vetch monoculture than in the mixtures at all N fertilization rates (Table 4). The N fertilization increased N_R concentration in oat monoculture, while in the mixtures or common vetch monoculture, the effect of fertilization was weakened. (Table 4). The P_R concentration increased with the proportion of common vetch and tended to be stimulated by N fertilization. Under N0, N50, and N150 fertilization, P_R concentration was significantly greater in VA21 than common vetch monoculture and VA12 (Table 4).

Table 3. Effects (*F* value) of mixing, fertilization, and their interaction on root C, N, and P concentrations and stoichiometric ratios.

Year	Treatment	C_R	N_R	P_R	$C{:}N_R$	$C{:}P_R$	$N{:}P_R$
2019	Mixing (M)	83.09 ***	212.69 ***	18.55 ***	104.66 ***	139.88 ***	28.68 ***
	Fertilization (F)	2.91	6.24 **	0.97	9.24 ***	22.59 ***	40.81 ***
	M × F	6.78 ***	11.34 ***	5.04 ***	16.50 ***	45.65 ***	27.03 ***
2020	Mixing (M)	0.81	152.72 ***	33.21 ***	64.75 ***	25.06 ***	4.78 **
	Fertilization (F)	1.50	10.85 ***	2.66 *	8.73 ***	3.46 *	4.89 **
	M × F	0.50	1.93 *	2.69 **	2.28 *	2.42 *	3.22 **

The C_R, N_R, and P_R represent root C, N, and P concentrations, respectively. The $C{:}N_R$, $C{:}P_R$, and $P{:}N_R$ represent root C:N, C:P, and P:N, respectively. The asterisks (*, ** and ***) show significant differences at $p < 0.05$, 0.01, and 0.001, respectively.

In 2020, N_R and P_R concentrations were significantly affected by mixing and fertilization and their interaction (Table 3). The N_R concentration increased with the proportion of common vetch and was significantly greater in common vetch monoculture than all other grasslands under all fertilization treatments (Table 4). In VA12 and oat monoculture, N_R concentration under P1 fertilization was significantly lesser than under all N fertilization treatments, while it was significantly lesser under P2 fertilization than under all N fertilization treatments.

Mixing, fertilization, and their interaction significantly affected $C{:}N_R$, $C{:}P_R$, and $N{:}P_R$ in 2019 and 2020 (Table 3). In 2019, $C{:}N_R$ increased with the decreasing proportion of common vetch under N0 and N50 fertilization (Figure 2a). In oat monoculture, $C{:}N_R$ was significantly reduced by N fertilization. (Figure 2a). The $N{:}P_R$ in oat monoculture was significantly greater than in other grasslands under N150 fertilization, while $N{:}P_R$ in common vetch monoculture was significantly greater than in other grasslands under N0 fertilization (Figure 2c).

In 2020, compared with no fertilization (CK), $C{:}N_R$ increased with decreasing proportion of common vetch under all fertilization treatments. The $C{:}P_R$ in oat monoculture was highest under all fertilization treatments (Figure 2e). In the mixtures, $C{:}P_R$ were stimulated by fertilization (Figure 2e). Compared with CK, $N{:}P_R$ under P1 and P2 fertilization hardly changed in oat monocultures, but under NP1 and NP2 fertilization, $N{:}P_R$ in oat monoculture was significantly higher.

Table 4. Root C, N, and P concentrations under different fertilization rates and mixing ratios in 2019 and 2020.

Year	Treatment	Organic C (mg/g)				Total N (mg/g)				Total P (mg/g)			
		V	VA21	VA12	A	V	VA21	VA12	A	V	VA21	VA12	A
2019	N0	550 ± 8 Ba	470 ± 4 Bb	488 ± 8 b	476 ± 5 Bb	19.9 ± 0.25 Ba	16.8 ± 0.33 Bb	14.4 ± 0.31 ABc	9.2 ± 0.14 Bd	1.62 ± 0.05 Ba	1.91 ± 0.14 a	1.48 ± 0.05 a	1.00 ± 0.02 Bb
	N50	551 ± 2 Ba	488 ± 8 ABb	475 ± 1 b	542 ± 1 Aa	21.6 ± 0.60 ABa	15.9 ± 0.31 Bb	13.2 ± 0.28 Bc	12.3 ± 0.09 Ac	1.81 ± 0.06 B	1.61 ± 0.15	1.31 ± 0.02	1.37 ± 0.06 A
	N100	588 ± 4 Aa	509 ± 6 Ab	465 ± 5 c	470 ± 4 Bc	22.4 ± 0.04 Aa	13.7 ± 0.13 Cbc	14.8 ± 0.11 Ab	13.6 ± 0.36 Ac	2.34 ± 0.12 Aa	1.45 ± 0.11 b	1.29 ± 0.08 b	1.40 ± 0.04 Ab
	N150	568 ± 6 ABa	496 ± 2 ABb	480 ± 4 b	484 ± 3 Bb	19.7 ± 0.41 Ba	18.6 ± 0.35 Aa	14.9 ± 0.23 Ab	13.2 ± 0.26 Ab	1.43 ± 0.10 Bb	1.99 ± 0.04 a	1.59 ± 0.05 b	0.80 ± 0.05 Bc
2020	CK	442 ± 12	438 ± 8	437 ± 3	423 ± 24	21.8 ± 0.08 Aa	12.2 ± 1.00 ABb	13.5 ± 0.03 Ab	9.0 ± 0.57 Bc	2.62 ± 0.04 a	1.63 ± 0.04 BCb	2.30 ± 0.11 Aa	1.50 ± 0.10 b
	P1	436 ± 4	453 ± 8	438 ± 2	443 ± 4	20.5 ± 0.60 Aa	13.0 ± 0.78 ABb	9.2 ± 0.49 Bc	8.1 ± 0.36 Bc	2.35 ± 0.02 a	1.27 ± 0.01 Cb	1.31 ± 0.06 Cb	1.25 ± 0.07 b
	P2	437 ± 3	434 ± 5	442 ± 5	426 ± 2	16.8 ± 0.33 Ba	11.2 ± 0.12 Bb	9.5 ± 0.28 Bc	8.6 ± 0.34 Bc	2.28 ± 0.15 a	2.12 ± 0.17 ABab	1.43 ± 0.11 Cbc	1.27 ± 0.08 c
	N	457 ± 2	432 ± 9	440 ± 6	434 ± 5	20.1 ± 0.56 Aa	13.5 ± 0.90 ABb	12.3 ± 0.52 Ab	11.8 ± 0.49 Ab	2.62 ± 0.17 a	1.61 ± 0.07 BCb	1.40 ± 0.07 Cb	1.37 ± 0.04 b
	NP1	456 ± 5	445 ± 8	444 ± 6	439 ± 10	21.6 ± 0.11 Aa	15.3 ± 0.49 Ab	12.7 ± 0.64 Ac	12.3 ± 0.14 Ac	1.96 ± 0.20 ab	2.24 ± 0.09 Aa	2.08 ± 0.24 ABab	1.35 ± 0.10 b
	NP2	448 ± 2	454 ± 3	448 ± 6	455 ± 6	19.6 ± 0.73 Aa	12.9 ± 0.21 ABb	12.1 ± 0.31 Ab	12.4 ± 0.73 Ab	2.59 ± 0.22 a	1.58 ± 0.06 BCb	1.69 ± 0.04 BCb	1.33 ± 0.09 b

Values are presented as mean ± S.E. ($n = 3$). Different capital letters show significant difference among fertilization rates under the same mixing ratio ($p < 0.05$). Different lowercase letters show significant difference among mixing ratios under the same fertilization rate ($p < 0.05$).

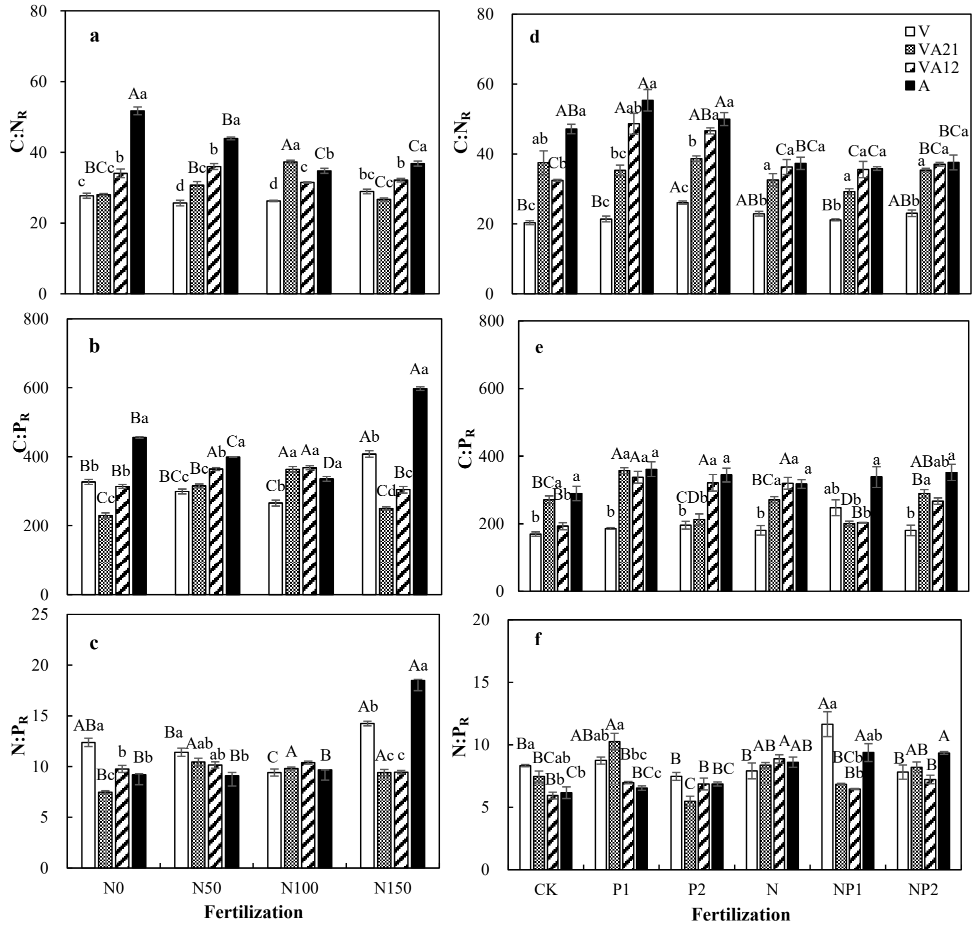


Figure 2. Root C, N, P stoichiometric ratios under different fertilization rates and mixing ratios in 2019 and 2020. (**a**–**c**) show 2019 data and (**d**–**f**) show 2020 data. Values are presented as mean ± S.E. (n = 3). Different capital letters show significant difference among fertilization rates under the same mixing ratio ($p < 0.05$). Different lowercase letters show significant difference among mixing ratios under the same fertilization rate ($p < 0.05$).

3.2. Soil C, N, P Contents and Stoichiometric Ratios

In 2019, C_S, N_S, and P_S contents were significantly affected by mixing and its interaction with fertilization (Table 5). The N fertilization significantly affected N_S content only in common vetch monoculture, and N_S content under N0 was significantly greater than N100 and N150 fertilization (Table 6). The P_S content was stimulated by fertilization in the mixtures, while it sharply decreased with N fertilization in common vetch monoculture.

Table 5. Effects (*F* value) of mixing, fertilization and their interaction on soil C, N, and P contents and stoichiometric ratios.

Year	Treatment	C_S	N_S	P_S	$C:N_S$	$C:P_S$	$N:P_S$
2019	Mixing (M)	6.70 **	4.34 *	28.69 ***	8.58 ***	20.21 ***	12.48 ***
	Fertilization (F)	1.48	0.45	14.15 ***	0.97	11.04 ***	12.31 ***
	M×F	7.02 ***	2.43 *	4.38 **	9.18 ***	6.96 ***	1.11
2020	Mixing (M)	11.41 ***	3.29 *	13.05 ***	1.46	9.45 ***	6.07 **
	Fertilization (F)	23.32 ***	19.98 ***	64.14 ***	16.83 ***	28.68 ***	23.48 ***
	M×F	22.81 ***	12.91 ***	76.81 ***	8.06 ***	22.51 ***	19.85 ***

The C_S, N_S, and P_S represent soil C, N, and P contents, respectively. The $C:N_S$, $C:P_S$, and $P:N_S$ represent soil C:N, C:P, and P:N, respectively. The asterisks (*, ** and ***) show significant differences at $p < 0.05$, 0.01, and 0.001, respectively.

Table 6. Soil C, N, and P content under different fertilization rates and mixing ratios in 2019 and 2020.

Year	Treatment	Organic C (mg/cm^2)				Total N (mg/cm^2)				Total P (mg/cm^2)			
		V	VA21	VA12	A	V	VA21	VA12	A	V	VA21	VA12	A
2019	N0	266 ± 9 Bb	310 ± 10 ab	261 ± 7 Bb	331 ± 10 Aa	33.8 ± 0.41 Aa	29.8 ± 0.31 b	32.2 ± 0.62 ab	31.1 ± 0.52 ab	28.6 ± 0.20 Aa	19.7 ± 0.22 ABc	21.1 ± 0.30 ABbc	22.4 ± 0.38 ABb
	N50	271 ± 4 Bb	293 ± 8 ab	330 ± 11 Aa	279 ± 10 Bb	33.0 ± 0.24 ABa	28.2 ± 0.38 b	32.3 ± 0.49 a	31.5 ± 0.56 a	28.4 ± 0.15 Aa	19.7 ± 0.67 ABc	21.2 ± 0.56 ABbc	23.5 ± 0.82 ABb
	N100	313 ± 10 ABa	318 ± 12 a	353 ± 7 Aa	222 ± 4 Cb	30.1 ± 0.58 C	31.9 ± 0.57	32.9 ± 0.73	31.9 ± 0.45	23.3 ± 0.54 Bab	21.2 ± 0.38 Ab	23.9 ± 0.64 Aab	26.4 ± 0.86 Aa
	N150	346 ± 13 Aa	299 ± 6 ab	343 ± 11 Aa	261 ± 4 BCb	30.7 ± 0.56 BC	30.6 ± 1.06	32.5 ± 0.47	34.7 ± 1.00	21.8 ± 0.56 Ba	18.2 ± 0.24 Bb	18.7 ± 0.79 Bab	20.5 ± 0.61 Bab
2020	CK	276 ± 4 Ba	269 ± 3 ABab	251 ± 5 Cb	283 ± 3 Aa	29.1 ± 0.44 Ba	26.7 ± 0.31 Bab	23.5 ± 0.91 Db	27.7 ± 0.62 ABCa	17.2 ± 0.05 Aa	15.3 ± 0.35 BCb	14.7 ± 0.01 Bb	17.9 ± 0.23 Ba
	P1	237 ± 6 CD	249 ± 4 BCD	264 ± 7 BC	255 ± 3 BC	24.7 ± 0.56 Cb	21.8 ± 0.16 Cc	27.8 ± 0.36 BCa	26.5 ± 0.32 BCab	15.8 ± 0.36 Ba	15.8 ± 0.27 BCa	15.6 ± 0.20 Ba	13.6 ± 0.09 Cb
	P2	223 ± 1 Db	284 ± 3 Aa	271 ± 4 BCa	287 ± 2 Aa	27.6 ± 0.33 Bbc	31.6 ± 0.64 Aa	25.7 ± 0.37 CDc	29.5 ± 0.19 Aab	13.9 ± 0.28 Cc	28.7 ± 0.23 Aa	15.2 ± 0.15 Bc	21.5 ± 0.30 Ab
	N	251 ± 3 Cb	243 ± 6 CDb	278 ± 0.25 Ba	248 ± 2 Cb	28.9 ± 0.04 Bb	30.5 ± 0.34 Aa	31.2 ± 0.42 Aa	28.2 ± 0.03 ABb	15.7 ± 0.08 Bb	16.4 ± 0.29 Bb	18.5 ± 0.38 Aa	12.1 ± 0.24 Dc
	NP1	369 ± 1 Aa	262 ± 4 BCc	280 ± 3 Bb	272 ± 3 ABbc	34.9 ± 0.53 Aa	27.3 ± 0.65 Bbc	29.6 ± 0.34 ABb	26.0 ± 0.46 Cc	17.3 ± 0.22 Aa	12.6 ± 0.08 Dc	15.9 ± 0.29 Bb	17.3 ± 0.18 Ba
	NP2	290 ± 2 Ba	231 ± 3 Db	303 ± 2 Aa	240 ± 5 Cb	27.7 ± 0.38 Bb	27.4 ± 0.39 Bb	32.1 ± 0.58 Aa	28.4 ± 0.35 ABb	14.9 ± 0.10 BCb	14.8 ± 0.22 Cb	18.8 ± 0.20 Aa	18.4 ± 0.49 Ba

Values are presented as mean ± S.E. ($n = 3$). Different capital letters show significant difference among fertilization rates under the same mixing ratio ($p < 0.05$). Different lowercase letters show significant difference among mixing ratios under the same fertilization rate ($p < 0.05$).

In 2020, C_S, N_S, and P_S contents were significantly affected by mixing, fertilization, and their interaction (Table 5). The P_S content were significantly higher in the monocultures than in the mixtures without fertilization (Table 6). Under N fertilization, C_S, N_S, and P_S contents increased first and then decreased with the decreasing proportion of common vetch, and in VA12 it was greater than other grasslands. The P fertilization significantly decreased C_S content in common vetch monoculture. The C_S content was significantly greater under N + P fertilization (NP1 and NP2) than P fertilization without N (P1, P2) in common vetch monoculture.

In 2019, mixing and its interaction with fertilization significantly affected C:N_S, while C:P_S and N:P_S were affected by mixing and fertilization, and C:P_S was also affected by their interaction (Table 5). The N fertilization increased C:N_S, C:P_S, and N:P_S in common vetch monoculture, while there was an opposite trend in oat monoculture (Figure 3a–c). The C:N_S and C:P_S in the mixtures were significantly greater than in common vetch monoculture under N50 fertilization, while greater than in oat monoculture under higher fertilization rates.

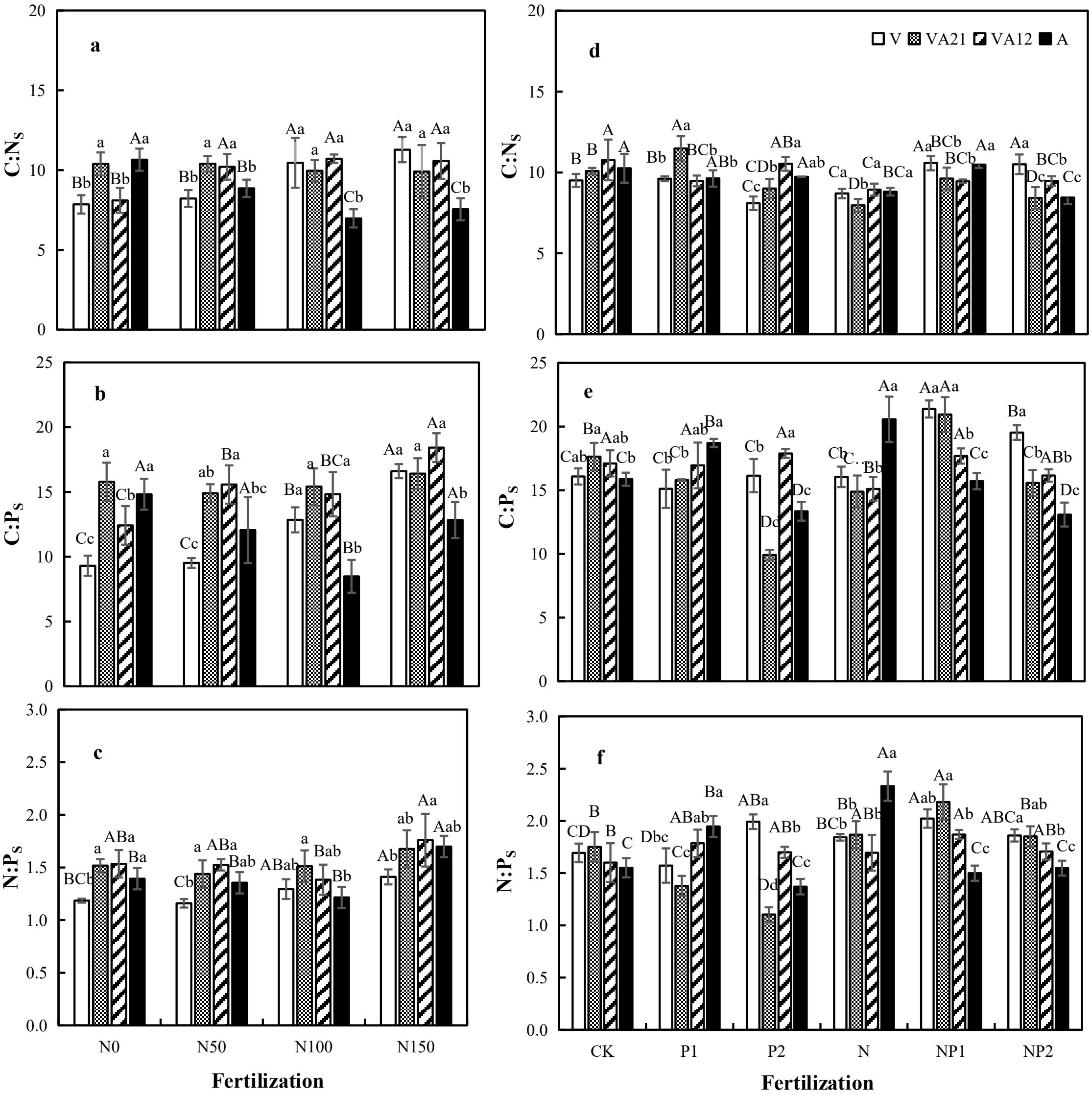
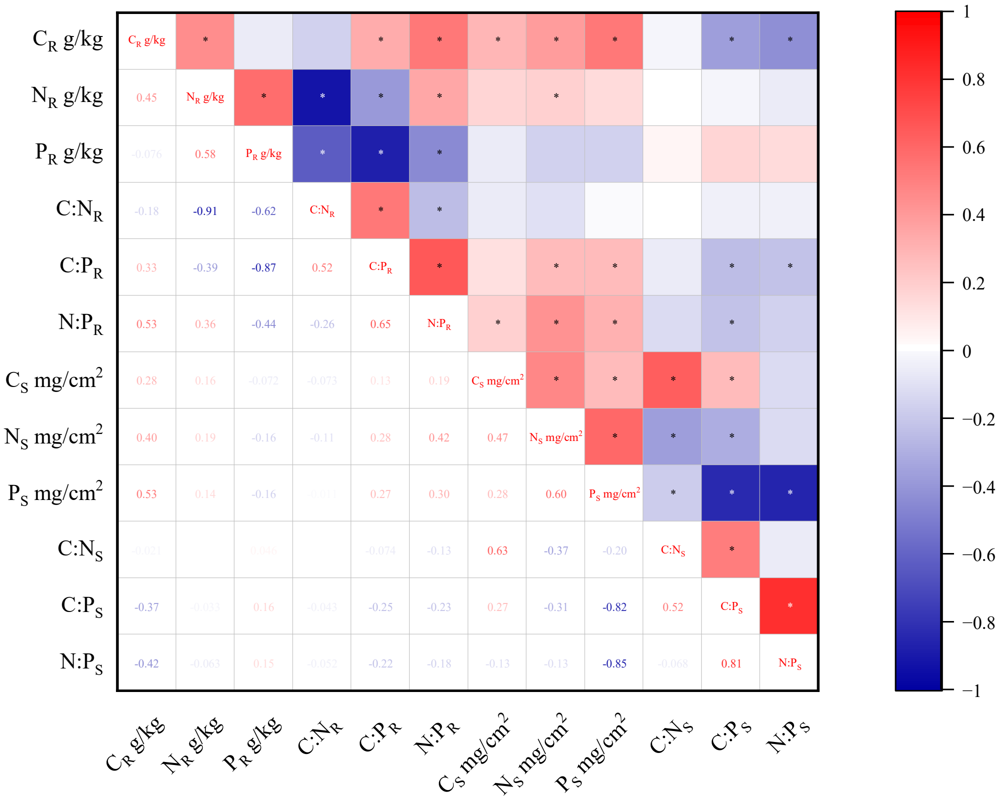


Figure 3. Soil C, N, and P stoichiometric ratios under different fertilization rates and mixing ratios in 2019 and 2020. (**a**–**c**) show 2019 data and (**d**–**f**) show 2020 data. Values are presented as mean ± S.D. (n = 3). Different capital letters show significant difference among fertilization rates under the same mixing ratio ($p < 0.05$). Different lowercase letters show significant difference among mixing ratios under the same fertilization rate ($p < 0.05$).

In 2020, mixing and fertilization and their interaction significantly affected C:P$_S$ and N:P$_S$, while C:N$_S$ was significantly affected by fertilization and its interaction with mixing (Table 5). The C:N$_S$ in common vetch monoculture and VA21 significantly decreased with the increase in P fertilization rate (Figure 3d). Compared with CK, N fertilization decreased C:N$_S$ significantly in all grasslands. The C:N$_S$ and C:P$_S$ (Figure 3e) in common vetch monoculture was increased by NP1 and NP2 fertilization and was significantly greater than other fertilization treatments.

3.3. Correlations of Nutritional Indexes between Root and Soil

The C$_R$ concentration was significantly positively correlated with C$_S$, N$_S$, and P$_S$ contents, while it was significantly negatively correlated with C:P$_S$ and N:P$_S$ (Figure 4). The N$_R$ concentration was significantly positively correlated with N$_S$ content. The C:P$_R$ was significantly positively correlated with N$_S$ and P$_S$ contents, but it was significantly negatively correlated with C:P$_S$ and N:P$_S$. The N:P$_R$ was significantly positively correlated with C$_S$, N$_S$, and P$_S$ contents.



	C$_R$ g/kg	N$_R$ g/kg	P$_R$ g/kg	C:N$_R$	C:P$_R$	N:P$_R$	C$_S$ mg/cm^2	N$_S$ mg/cm^2	P$_S$ mg/cm^2	C:N$_S$	C:P$_S$	N:P$_S$
C$_R$ g/kg	C$_R$ g/kg	*			*	*	*	*	*		*	*
N$_R$ g/kg	0.45	N$_R$ g/kg	*	*	*	*		*				
P$_R$ g/kg	-0.076	0.58	P$_R$ g/kg	*	*	*						
C:N$_R$	-0.18	-0.91	-0.62	C:N$_R$	*	*						
C:P$_R$	0.33	-0.39	-0.87	0.52	C:P$_R$	*		*	*		*	*
N:P$_R$	0.53	0.36	-0.44	-0.26	0.65	N:P$_R$	*	*	*		*	
C$_S$ mg/cm^2	0.28	0.16	-0.072	-0.073	0.13	0.19	C$_S$ mg/cm^2	*	*	*	*	
N$_S$ mg/cm^2	0.40	0.19	-0.16	-0.11	0.28	0.42	0.47	N$_S$ mg/cm^2	*	*	*	
P$_S$ mg/cm^2	0.53	0.14	-0.16	-0.011	0.27	0.30	0.28	0.60	P$_S$ mg/cm^2	*	*	*
C:N$_S$	-0.021		0.046		-0.074	-0.13	0.63	-0.37	-0.20	C:N$_S$	*	
C:P$_S$	-0.37	-0.033	0.16	-0.043	-0.25	-0.23	0.27	-0.31	-0.82	0.52	C:P$_S$	*
N:P$_S$	-0.42	-0.063	0.15	-0.052	-0.22	-0.18	-0.13	-0.13	-0.85	-0.068	0.81	N:P$_S$

Figure 4. Correlations of nutritional indexes between root and soil. * Signifies significance. The value represents the correlation coefficient (r). The color column represents the range of correlation coefficients.

4. Discussion

4.1. Effect of Mixing and Fertilization on Root C and Nutrients

In this study, C$_R$, N$_R$, and P$_R$ concentrations were significantly affected by mixing and increased with increasing common vetch proportion in the mixtures. It is consistent with previous studies that legumes have higher nutrient concentrations in fine roots than non-legume plants [24,25]. The high N$_R$ concentration may be partly due to legume BNF which provides a promising N source for the plant [26]. Moreover, legumes have greater root phosphatase activity than non-legumes [27,28], which leads to more available P in soils and more P uptake by the plant. Therefore, with the increase in common vetch proportion, nutrients accumulated in the bulk root of the whole mixing system. The N:P$_R$ showed no significant change with mixing ratio, while C:N$_R$ and C:P$_R$ decreased with the increase in legume proportion in the mixtures. This is because C$_R$ concentration increased but with a lower rate than N_R and P_R concentrations. Additionally, lower nutrient use efficiency also implies there is relatively adequate N and P availability in the mixtures. There is a significant negative correlation between root decomposition and C_R concentration [18]. It is generally accepted that lower nutrient use efficiency, that is lower $C:N_R$ and $C:P_R$, results in higher decomposition rate and soil nutrient cycling rate [29]. This may finally lead to higher levels of soil C accumulation due to rapid decomposition and return of organic matter. Extensive exploration is needed to address this point.

In this study, fertilization had a significant effect on N_R concentration, but barely affected C_R and P_R concentrations. The C is mainly a structural substance and generally remains steady within a plant. In contrast, the absorption and utilization of N and P are more complex [30]. The N fertilization can hopefully promote soil P mobilization due to soil acidification [28] and P fertilization should directly increase soil P availability. Both ways would have led to increased uptake of soil available P. However, in this study P_R concentration hardly changed in response to fertilization. It is assumed that the so called "dilution effect" due to accelerated growth under fertilization would have reduced plant P and P_R concentration, which is finally balanced by increased P uptake. In this study, N or N + P fertilization significantly promoted N_R concentration, as well as $N:P_R$. It is reasonable that N fertilization leads to increased soil mineral N content, which in turn increases plant N uptake and accumulation. A past study also showed that there was no effect of nitrate addition on N_R concentration in the wetland [31]. This may be attributed mainly to the antagonistic effect of N fertilizer on legume BNF, which finally affects N uptake and accumulation. Compared with no fertilization, P fertilization led to lower N_R concentration. The N_R concentration under P1 and P2 was 11.1% and 19.2% less than CK, respectively. The increased P availability due to P fertilization possibly leads to the relative N limitation to the growth in grasslands [17]. From another aspect, soil available P may promote BNF due to facilitated bacteria activity [32], which consequently leads to easier and more N uptake. This should balance a little N_R concentration reduction but might be not strong enough. Therefore, N_R concentration was lesser under P fertilization in this study.

4.2. Effects of Mixing and Fertilization on Soil C and Nutrients

In this study, C_S content barely changed with increasing legume proportion. This is consistent with general knowledge that soil C storage remains steady during a short term [33]. It also suggested that mixing common vetch and oat imposes no significant impact on C_S content in the experimental duration. From another viewpoint, during the growth, dead root may continuously decompose to return organic matter to the soil, which can potentially change C_S content [12]. The return of root litter with low C:N increases the energy requirement (e.g., in the form of C) of microorganisms, resulting in more C being processed and accumulated [34]. Therefore, the return of low C:N root may finally lead to more microbial C accumulation in soils and increased soil C storage. However, we failed to observe such C_S content increase with increasing legume proportion. It is assumed that during the growth of these two annuals, fertilization (N fertilization) may accelerate the decomposition of root litter [35], and more C in the litter may be exhausted by microorganisms, which leads to less C return into the soil and less use of the original soil organic matter. So, the effect of increasing legume proportion on soil C storage was ameliorated. Generally, legumes tend to significantly increase N accumulation in soils [8]; however, in this study N_S content did not show an increasing trend with the increase in legume proportion. Under all fertilization treatments, N_S content in VA21 (common vetch proportion 62.5%) was lesser than that in VA12 (common vetch proportion 29.4%). When the proportion of legumes is higher than that of non-legumes, soil N storage is reduced in a temperate steppe grassland [8]. When the density of legume increases, the intraspecific competition is intensified, resulting in the weakness of interaction between non-legume and legume in N utilization and the decrease in N transfer from the legume [9]. Therefore, the utilization of soil N by both plants increases and the accumulation of soil N is largely inhibited. Additionally, this also led to N_R concentration increase with increasing common vetch proportion. In contrast, change in P_S content with common vetch proportion was affected by fertilization. Under N or N + P fertilization, P_S content in VA12 was greater than in VA21, while it was opposite under only P fertilization. The N fertilization leads to soil acidification [28] and in turn release of fixed P, which contributes to increase P_S content. However, legumes usually have a higher P demand because of BNF [36]. Therefore, more common vetch individuals should use more soil P, lowering P_S content more in VA21. The P supply benefits the growth of legume, resulting in more secretion of phosphatase which solubilizes more P in soils. Therefore, with a high proportion of common vetch there was more available P in the soil.

In this study, N or N + P fertilization tended to increase N_S and P_S contents. However, higher levels of N fertilization tended to decrease N_S and P_S contents. Compared with no fertilization, N150 led to decrease in N_S and P_S content by 12.1% and 13.5%, respectively. Excess N supply leads to a strong antagonistic effect on BNF [7] and very rapid growth. In this case N_S and P_S contents were reduced and their availability cannot catch up with the requirement. The N input significantly enhanced soil C storage [35]. Compared with no fertilization, N or N + P fertilization tended to decrease soil C:N (C:N_S). The C:N_S is an auxiliary index that can reflect soil fertility, which contains a close conversion relationship between the accumulation and consumption of C and N [37]. In this study, averaged C:N_S was 9.5, lesser than the global value (13.33) and the value (10~12) of China [38]. This is possibly because of decomposition of roots with lower C:N. A lower C:N_S is beneficial for N mineralization and inhibited soil C fixation. The C:P_S and N:P_S were significantly affected by mixing and fertilization. The fertilization tended to decrease C:P_S, suggesting that net mineralization rate of soil P increased [39]. The P fertilization decreased N:P_S, while under N or N + P fertilization, N:P_S was increased. All of these may be partly attributed to the increase in available N and/or P content in soils due to fertilization.

4.3. Correlations of C and Nutrients between Root and Soil

Previous studies have shown a strong relation between C, N, and P in plants and soils [40,41]. On one hand, plant nutrients are affected by the availability of soil nutrients [42,43]. Therefore, soil nutritional status in the habitat largely controls the survival, growth and production of plants. On the other hand, the stability of plant C:N:P stoichiometry affects soil C and nutrients [41], possibly through litter decomposition and root exudate [15]. Root associated C is one of the main contributors to soil C storage [44]. Returning organic matter through roots is closely related to soil nutrients [18]. In this study, N_R concentration was significantly positively correlated with N_S content, showing the close and positive interaction between plant and soil; this is consistent with the study by Ma et al. (2015) [45]. However, Liu et al. (2019) found that legume N_R concentration shows no significant correlation with N_S content [25]. We are not sure if it is because of various sampling dates/stages. Moreover, the root and soil N:P was negatively correlated with C:P, which is inconsistent with previous studies in a semiarid grassland ecosystem [46] and in desert grasslands [47]. These differences may be attributed to root uptake and utilization of nutrients being related to soil N and P availability, not the soil N or P pool [25].

5. Conclusions

A higher proportion of common vetch in the grasslands promoted root C and nutrients. Root N concentration was promoted by N or N + P fertilization, but inhibited by P fertilization alone. Soil nutrients barely changed with mixing ratio, which was stimulated by fertilization. There was a close correlation in C and nutrients between root and soil, indicating the return of organic matter by roots into soils. This study will be helpful for further understanding of the coupling between root and soil, providing the scientific basis on the extensive use of common vetch–oat mixture in the Loess Plateau of China.

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Conflicts of Interest: We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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