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Effects of *Epichloë* endophyte and repeated cutting on nutrition compositions of *Festuca sinensis*

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Abstract: The presence of *Epichloë* endophyte can promote plant growth and increase the accumulation of host plant nutrients. We determined the dry matter (DM) and important nutritional indicators of E+ (infected by endophyte) and E– (not infected by endophyte) *Festuca sinensis* under the three-time repeated cutting. The results indicated that the total nitrogen, total phosphorus, crude protein (CP), crude fat (CF), crude ash (CA) contents, and DM of *F. sinensis* decreased with the repeated cutting increase and reached the minimum after the third time cut. The total organic carbon content of *F. sinensis* peaked at the second time cut. In addition, the DM of *F. sinensis* was significant ($P < 0.05$) positively correlated with its quality CP, CF, and CA contents, and the appropriate repeated cutting times of *F. sinensis* was 1–2 times. We concluded that the presence of endophyte and proper cutting frequency can increase the quality and biomass of *F. sinensis* in Western China.

Keywords: symbioses; grassland management; forage quality; perennial grass; agronomic performance

The *Epichloë* endophytes live in symbioses with host grasses. When the plant is in the vegetative state, the fungus causes no disease symptoms, and in some well-studied systems, it is apparent that the fungus provides a number of fitness enhancements (Schardl 2001, Schardl et al. 2009). They are an important component that colonises in healthy tissues of living plants and can be readily isolated from any microbial or plant growth medium (Li et al. 2017, White et al. 2019). Endophytes interact mutualistically with their host plant, mainly by enhancing the host plant resistance to biotic and abiotic stresses (Panaccione et al. 2014, Kowalski et al. 2015). The *Epichloë* endophyte and cutting are important factors affecting native grassland management efficiency of China (Nan 1996, Zhang et al. 2018).

Appropriate cutting can promote grasses regeneration, increase dry matter, and improve nutrition value. Previous studies have shown that cutting times and height can significantly alter plant dry matter accumulation and impact the compositions of grasses (Aldous 1930, Zhong and Zhong 2007, Trócsányi et al. 2009).

Festuca sinensis is an important perennial grass species that is distributed across the semi-arid regions of China (Lin et al. 2018, 2019). It's often infected by an asexual, symptomless *Epichloë* endophyte (Zhou et al. 2015). Its advantages include good agronomic performance and high yields (Tian et al. 2015); the crude protein and crude fat contents are 13.57% and 2.38%, respectively (Sun 2008). Liu et al. (2015) showed that cutting reduces the crude protein content of *F. sinensis*. Nan (1996) found that the presence of endophyte promoted the growth of *F. sinensis*. Wang et al. (2017) reported that the endophyte enhanced *F. sinensis* resistance to drought and waterlogged

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conditions. To the best of our knowledge, there is no report about harmfulness in animals grazing *F. sinensis* infected by endophyte, signifying that the potential finding of "mammalian-safe" nontoxic grasses-endophytes associations (Tian et al. 2018). Nonetheless, it remains unclear whether endophyte affects the quality compositions of *F. sinensis* under repeated cutting.

By performing a series of experiments under greenhouse conditions, we determined the total organic carbon (TOC); total nitrogen (TN); total phosphorus (TP); crude protein (CP); crude fat (CF); crude ash (CA), and aboveground dry matter (DM) of E+ (infected by endophyte) and E– (not infected by endophyte) *F. sinensis* under the different repeated cutting. This study explored whether the presence of endophyte could increase the nutrient compositions of *F. sinensis* under repeated cutting.

MATERIAL AND METHODS

Plant materials. Seeds of *F. sinensis* Keng ex E. B. Alexeev were collected from natural grasslands of Hongyuan county, Sichuan province, China (102°33'E, 32°48'N, altitude 3 491 m a.s.l.) in the summer of 2013. After collection, seeds were stored at a constant temperature of 4 °C at Lanzhou University, China (Tian et al. 2018).

Experimental design. In May 2017, healthy and well-filled seeds were planted into a 72-hole plastic seedling tray containing sterilised peat soil (https://mjlcaotantu.cdgtw.net) in a greenhouse at the College of Pastoral Agriculture Science and Technology, Yuzhong campus of Lanzhou University. The trays were watered as required. Two months after sowing, the presence of endophyte in the seedlings was determined by performing the microscopic examination of host leaf-sheath samples after stained with aniline blue (Nan 1996, Lin et al. 2019). The E+ and E– seedlings were marked and transplanted to the pots with the random position in the greenhouse. The pots (diameter: 25 cm; height: 20 cm) were filled with 1.5 kg of peat soil. They were maintained at a constant temperature (24 ± 2 °C) with natural daylights. Each E+ and E– plants had five replications.

After three months of growth, the plants were cut on 29 October 2017 (first-time cut), and then the plants were repeated cut another two times, which were on 27 December 2017 (second-time cut) and 30 March 2018 (third-time cut). The plants were cut at 4 cm above the soil surface, and the cut materials were collected. All harvest plant samples were freeze-dried (PowerDry LL 3000, Thermo Fisher Scientific, Waltham, USA) to determine the dry matter. Subsequently, the plant samples were ground into powder in a mixer mill (MM 400; Retsch, Haan, Germany) for 2 min at 30 Hz for analysing the nutrition composition.

Determination of nutrient composition. Plant total organic carbon content was measured using the K_2CrO_7-H_2SO_4 oxidation method (oil bath at 180 °C for 5 min, followed by titration with $FeSO_4$) (Tanveer et al. 2014). Total nitrogen and phosphorus contents in samples were determined following digestion with H_2SO_4 at a temperature of 420 °C. The TN and TP contents in the digested solutions were determined by flow injection analysis using a FIAstar 5000 Analyser (FIAStar 5000, Foss Tecator, Hoganas, Sweden) (Xia et al. 2018). Crude protein (CP:N × 6.25); crude fat content was measured using diethyl ether extracted method (samples were extracted for a minimum of 6 h with diethyl ether) (De Boever et al. 1996). Crude ash content was obtained after incineration at 600 °C (De Boever et al. 1996).

Data analysis. Statistical data analysis was performed with SPSS Inc. (Released 2009, PASW Statistics for Windows, version 18.0. Chicago, USA, SPSS Inc). Effects of cutting, endophyte, and interaction between endophyte and cutting on the TOC, TN, TP, CF, CP, CA contents, and DM were evaluated through a two-way analysis of variance (ANOVA). A one-way ANOVA was used to analyse the significant differences of the same endophyte situations (E+ or E–) *F. sinensis* under different cutting conditions. Significance differences between E+ and E– plants were determined by independent-sample *t*-tests. Statistical significance was defined at the 95% confidence level. Means are reported with their standard errors. Using Pearson correlation analysed the correlations among TOC, TP, CF, CP, CA contents, and DM.

RESULTS

TOC. Endophyte didn't have significant effects on the TOC contents of *F. sinensis*, whereas the repeated cutting had significantly ($P < 0.05$) effects on them (Table 1). The TOC contents of E+ and E– *F. sinensis* peaked at second time cut, which were significantly ($P < 0.05$) higher than those of the first and third-time cut (Figure 1A). There was no significant difference between the first and the third-time cut (Figure 1A).

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Table 1. Results of two-way ANOVA for the effects of cutting and endophyte on total organic carbon (TOC), total nitrogen (TN), and total phosphorus (TP) of *Festuce sinensis* at $P < 0.05$ level

Treatment	*df*	TOC		TN		TP	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Cutting	2	10.842	< 0.01	156.965	< 0.01	16.243	< 0.01
Endophyte	1	0.107	0.746	56.359	< 0.01	26.527	< 0.01
Cutting × endophyte	2	0.74	0.488	7.726	0.003	11.632	< 0.01

TN. Both endophyte and cutting significantly ($P < 0.05$) affected the TN contents of *F. sinensis* (Table 1). The presence of endophyte significantly ($P < 0.05$) increased the TN contents of *F. sinensis* after the first and third-time cut, with a relative increase of 11.7% and 30.0%, respectively (Figure 1B). The TN contents of E+ and E– *F. sinensis* significantly ($P < 0.05$) decreased after the third time cut, which were sig-

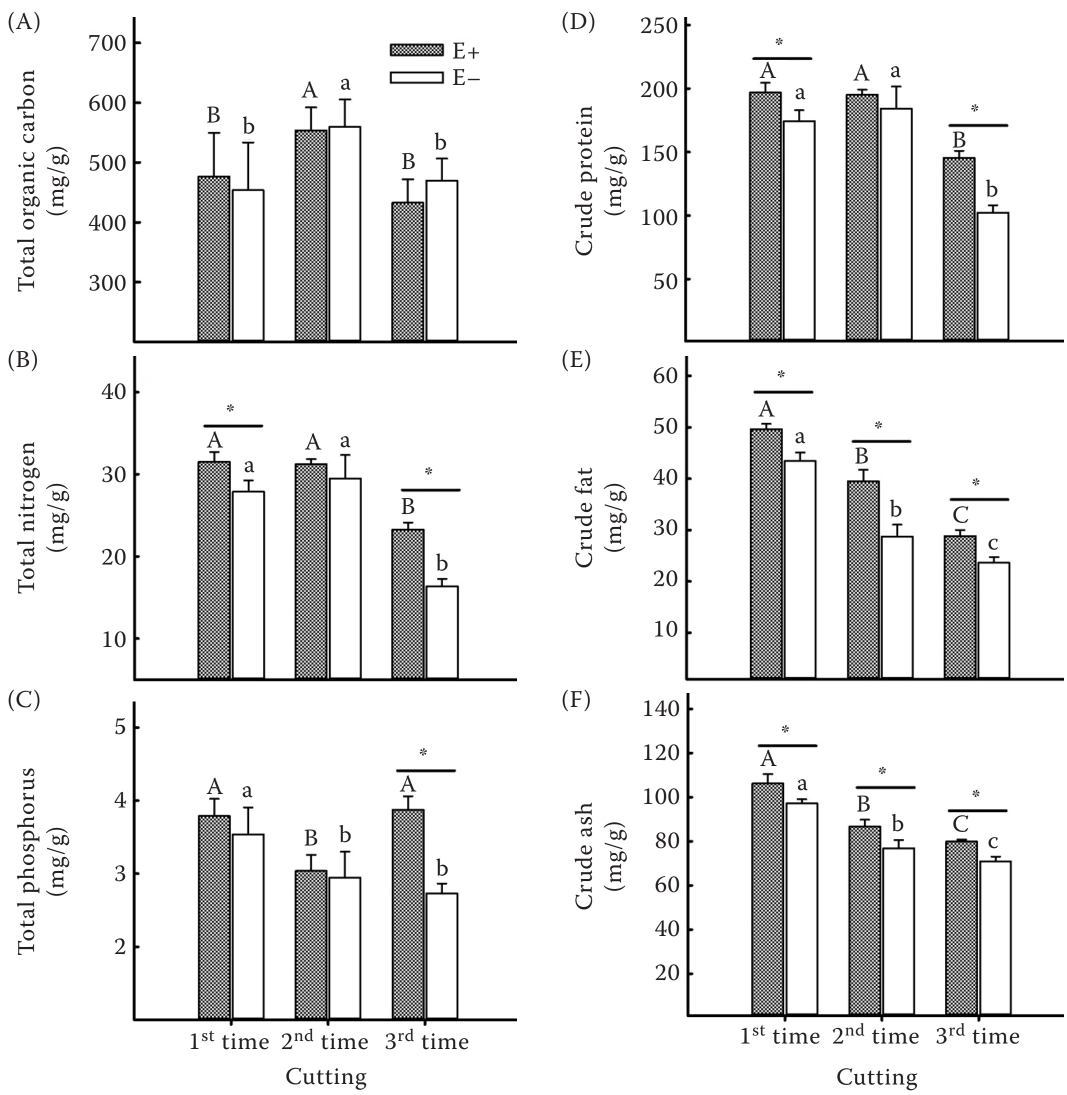

Figure 1. Effects of cutting and endophyte on the total organic carbon, total nitrogen, total phosphorus, crude protein, crude fat and crude ash contents of *Festuca sinensis*. Values are the mean ± standard error. *Represents a significant difference between E+ (infected by endophyte) and E– (not infected by endophyte) at $P < 0.05$ level. Different upper case letters indicate significances among repeated cutting conditions for E+ *F. sinensis* at $P < 0.05$ level, different lower case letters indicate significances among repeated cutting conditions for E– *F. sinensis* at $P < 0.05$ level

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Table 2. Results of two-way ANOVA for the effects of cutting and endophyte on crude protein (CP), crude fat (CF), crude ash (CA), and dry matter (DM) of *Festuca sinensis* at $P < 0.05$ level

Treatment	*df*	CP		CF		CA		DM	
		F-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value	*F*-value	*P*-value
Cutting	2	157.3	< 0.01	373.783	< 0.01	216.85	< 0.01	11.739	< 0.01
Endophyte	1	56.63	< 0.01	146.261	< 0.01	73.946	< 0.01	31.643	< 0.01
Cutting × endophyte	2	7.698	0.003	7.744	0.003	0.075	0.928	1.978	0.16

nificantly lower than them after the first and second cut (Figure 1B). There was no significant difference between the first and second time (Figure 1B).

TP. Both endophyte and cutting significantly ($P < 0.05$) affected the TP contents of *F. sinensis* (Table 1). The presence of endophyte significantly ($P < 0.05$) increased the TP content of *F. sinensis* after the third time cut, with a relative increase of 29.5% (Figure 1C). The TP contents of E+ and E– *F. sinensis* significantly ($P < 0.05$) decreased after the second time cut, which were significantly lower than them after the first cut (Figure 1C). The third time cut significantly ($P < 0.05$) reduced the TP content of E– *F. sinensis*, but it had no significant ($P > 0.05$) effect on the TP content of E+ *F. sinensis* (Figure 1C).

CP. Our results indicated that both endophyte and cutting significantly ($P < 0.05$) affected the CP contents of *F. sinensis* (Table 2). The presence of endophyte significantly ($P < 0.05$) increased the CP contents of *F. sinensis* after the first and third time cut, with a relative increase of 11.5% and 29.6%, respectively (Figure 1D); the CP contents of E+ and E– *F. sinensis* significantly ($P < 0.05$) decreased after the third time cut which were significantly lower than them after the first and second cut (Figure 1D). There was no significant difference between the first and second time (Figure 1D). The CP content and DM of E+ and E– *F. sinensis* showed a significant ($P < 0.01$) positive correlation under the repeated cutting (Table 3).

CF. The analysis results showed that both cutting and endophyte significantly ($P < 0.05$) affected the CF contents of *F. sinensis* (Table 2). The presence of endophyte significantly ($P < 0.05$) increased the CF content of *F. sinensis* after three repeated cutting, with a relative increase of 12.5, 27.2, and 18.2%, respectively (Figure 1E). The CF contents of E+ and E– *F. sinensis* decreased significantly ($P < 0.05$) with the increase of repeated cutting (Figure 1E). The CF, TP content, and DM of E+ and E– *F. sinensis* showed a significant ($P < 0.01$) positive correlation under the repeated cutting (Table 3).

CA. The analysis results showed that both cutting and endophyte significantly ($P < 0.05$) affected the CA contents of *F. sinensis* (Table 2). The presence of endophyte significantly ($P < 0.05$) increased the CA content of *F. sinensis* after three repeated cutting, with a relative increase of 8.5, 11.1, and 11.3%, respectively (Figure 1F). The CA contents of E+ and E– *F. sinensis* decreased significantly ($P < 0.05$) with the increase of repeated cutting (Figure 1F). The CA, TP content, and DM of E+ and E– *F. sinensis* showed a significant ($P < 0.01$) positive correlation under the repeated cutting (Table 3).

DM. The results showed that endophyte and cutting significantly ($P < 0.05$) affected the DM of *F. sinensis* (Table 2). The presence of endophyte significantly ($P < 0.05$) increased the DM of *F. sinensis* after the second and third time cut, with a relative increase of 10.4% and 27.9%, respectively (Figure 2); the DM of E+ and E– *F. sinensis* significantly ($P < 0.05$) decreased after the third time cut which were significantly lower than them after the first and second cut (Figure 2). There was no significant difference between the first and second time (Figure 2).

DISCUSSION

The C, N, and P are basic elements of plants, and their dynamics are very important in aquatic and

Table 3. Pearson correlation among dry matter (DM), nutrient elements (total phosphorus (TP) and total organic carbon (TOC)), and quality indicators (crude protein (CP), crude fat (CF), and crude ash (CA)) of *Festuca sinense*

DM and nutrient element	Quality indicator		
	CP	CF	CA
DM	0.678**	0.667**	0.654**
TP	0.2479	0.477**	0.578**
TOC	0.3303	–0.0134	–0.1295

**$P < 0.01$

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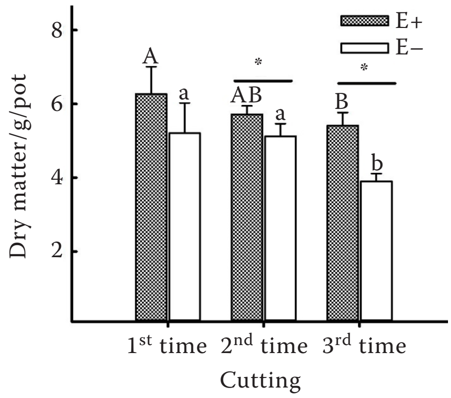

Figure 2. Effects of cutting and endophyte on the dry matter (DM) of *Festuca sinensis*. Values are the mean ± standard error. *Represents a significant difference between E+ (infected by endophyte) and E– (not infected by endophyte) at $P < 0.05$ level. Different upper case letters indicate significances among repeated cutting conditions for E+ *F. sinensis* at $P < 0.05$ level, different lower case letters indicate significances among repeated cutting conditions for E– *F. sinensis* at $P < 0.05$ level

terrestrial ecosystems (Vrede et al. 2004). Previous studies have shown that endophyte can promote the growth of host grasses with increases the accumulation of C, N, and P in grasses (Chen et al. 2018, Malinowski and Belesky 2019). Endophyte can increase net photosynthetic rates that the E+ plants had increased net photosynthetic rates, higher carbohydrate accumulation than E– plants under biotic or abiotic stress (Rozpadek et al. 2015). The results of the present study indicated that the presence of endophyte significantly increased the TN and TP contents of *F. sinensis* after different times cutting, but it had no significant effect on TOC contents of *F. sinensis.* Endophytic fungi are an important component that colonises in healthy tissues of living plants and grows in the stem base portion and tiller blades of plants (Nazir and Rahman 2018). During the cutting process, endophyte mycelium remains in the plant stem base portion and does not disappear with the cutting. Our results indicated that overcutting led to the decrease of the accumulation of C, N, and P in the tissue, and the presence of endophyte was conducive to N and P accumulation.

The CP, CF, and CA contents are three important indicators of forage quality. Previous studies showed that the forage quality of *Avena sativa* was higher at the second time cut than the first time cut, and the CP and CA contents of *A. sativa* were higher at the second time cut than the first time cut (Liu and Zhao 2006). Due to the grass cultivars being different, their quality also showed differences after different repeated cutting. The crude protein content per unit area of *A. sativa* (Bayan *N.3*) at cut once a year was 57.57% higher than cut twice a year (Liu and Zhao 2006, Zhu et al. 2009). Whereas crude protein content per unit area of *A. sativa* (Canada *N.4632*) at cut once a year was 53.68% lower than cut twice a year (Liu and Zhao 2006, Zhu et al. 2009). Our study indicated that the CP, CF, and CA contents of *F. sinensis* decreased with the increase of cutting times, which showed the forage quality decreased. Previous studies showed that endophyte infection can provide benefits to the host plant, e.g., enhancing drought tolerance, photosynthetic rate, and growth rate, so that the host can accumulate more compounds (Clay and Schardl 2002, Xia et al. 2018). The results of our study showed that the presence of endophyte significantly increased the CP, CF, and CA contents of *F. sinensis.* This indicated that endophyte could improve the quality of *F. sinensis* under the same cutting conditions.

There are reports about the beneficial impacts of seed priming with endophyte on the growth of *F. sinensis* under the greenhouse environment, which significantly improve aboveground dry weight and number of leaves of seedlings (Peng et al. 2013, Lin et al. 2018). Another study also discovered the beneficial effects of endophyte on plant growth and seedling biomass of *F. sinensis*-endophyte association under water-stressed environments (Wang et al. 2017). Our study results showed that the presence of endophyte significantly increased the DM of *F. sinensis* under the treatment of the second and third time cut, but it had no significant effect on DM of *F. sinensis* under the first time cut. This indicated that the repeated cutting enhanced the positive effect of endophyte on *F. sinensis*. Xia et al. (2018) showed that endophyte increased the dry matter of drunken horse grass (*Achnatherum inebrians*) under drought stress. Zhang et al. (2012) showed that endophyte increased the dry matter, tiller number, and germination rate of *Elymus dahuricus* under heavy metal (high concentrations Cd^{2+}) stress. These studies have demonstrated that endophyte can increase host biomass under abiotic stress. In addition, cutting is also an important factor affecting the biomass and compositions of grasses (Zhao et al. 2007). The results of the present study indicated that the third time cut significantly reduced the DM of *F. sinensis*, but the second time cut had no significant effect on DM of *F. sinensis.*

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Plant aboveground DM accumulates as the plant grows. After each cutting, the plant's aboveground DM increases with growth, but overcutting times led to the plant's growth rate to slow down, so its DM decreases (Zhang et al. 2018). In addition, the results of this study also showed that the DM of *F. sinensis* was positively correlated with its quality CP, CF, and CA contents. This means that the proper number of cutting times can harvest higher quality and more DM of *F. sinensis.*

The accumulation decreased of DM and TN, TP, CP, CF, and CA compositions of *F. sinensis* in the third time repeated cutting, but there was no significant difference between the first and second repeated cuttings. In summary, the appropriate repeated cutting times of *F. sinensis* was 1–2 times. The presence of endophyte promoted the accumulation of DM and TN and TP compositions in *F. sinensis*. This means that using E+ *F. sinensis* seeds will obtain greater economic benefits when planting *F. sinensis*. We concluded that the presence of endophyte and proper cutting frequency can increase the quality and biomass of *F. sinensis* in Western China.

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