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REGULAR ARTICLE

Epichloë gansuensis endophyte-infection alters soil enzymes activity and soil nutrients at different growth stages of *Achnatherum inebrians*

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Abstract
Background and Aims Epichloë endophytes are a unique model system for the study of the linkages between organisms above and belowground in ecosystems. However, the impact of *Epichloë gansuensis* in *Achnatherum inebrians* on soil enzymes activity and soil nutrients remains poorly understood.
Methods Achnatherum inebrians with (E+) and without *E. gansuensis* (E−) were established in different plots of an experimental field in 2011. At 2016, the activity of soil invertase, soil urease, soil alkaline phosphatase and the soil nutrient of E+ and E− plots was determined in the *A. inebrians* growth and senescent stages; further, we used NMDS to evaluate the roles of endophyte and growth phase for each sample with soil properties.
Results The interaction of *E. gansuensis*-infection and growth phase significantly influences TN and AN. *E. gansuensis* enhanced the activity of soil invertase and soil alkaline phosphatase in growth and senescence stages of *A. inebrians*, *E. gansuensis* increased the activity of urease in the growth stage. *E. gansuensis* significantly increased the content of soil TN and AN in the *A. inebrians* growth stage. The results of NMDS showed that *E. gansuensis* and growth phase significantly influenced the soil properties, respectively. Therefore, *E. gansuensis* played an important role in

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improving soil enzymes activity and soil nutrients content.

Conclusions E. gansuensis improve soil fertility through regulating soil enzymes activity and soil nutrients. This study provided insights into a poorly understood ecosystem function of *Epichloë* and enhanced knowledge of the benefits provided by these mutualistic fungal endophytes of many grass species.

Keywords *Achnatherum inebrians* · *Epichloë gansuensis* · Soil enzymes activity · Soil nutrient · Stoichiometry · Growth stage and senescent stage

Introduction

With the development of agriculture, overuse of pesticides and chemical fertilizers seriously affects food safety and soil physicochemical properties (Carvalho 2006; Iii et al. 2002; Riah et al. 2014). The excessive application of chemical fertilizers not only detrimentally influences soil physicochemical properties and soil nutrient status but also exacerbates ecosystem pollution (Nosheen et al. 2018). Meanwhile, there is growing interest in understanding the activity of soil-located enzymes and the important role of and on the soil biota (Burns et al. 2013).

Soil-located enzymes were important in soil nutrient cycling and modulate the functions of ecosystems (Makoi and Ndakidemi 2008). Soil organic matter, different plant species, the seasons, soil temperature and moisture can affect activity of enzymes in soil (Hassan et al. 2015; Machmuller et al. 2016; Razavi et al. 2017). Interestingly, soil-located enzymes play a central function in the soil system, especially during the organic matter decomposition process (Sinsabaugh et al. 1991). In additional, soil enzymes are central in catalyzing some vital reactions necessary for the soil life processes, and they contribute to organic matter formation, the decomposition of organic wastes and nutrient cycling. Soil enzymes strongly influence the soil fertility, environment and transformation (Dick et al. 1994; Nosheen et al. 2018). A study demonstrated that invertase activity depends on the presence of soil organic matter, and invertase plays a key role in carbon cycling and hydrolysis of sucrose into glucose (Shi et al. 2008; Wang et al. 2013). Further, urease is another important soil enzyme, and enhances the utilization efficiency of nitrogen fertilizer by hydrolysis of urea (Maithani et al. 2017). Another enzyme, soil phosphatase, plays a key role for the solubilization of phosphate and inorganic phosphate release, and provides usable phosphate to the plants (Behera et al. 2017; Satyaprakash et al. 2017).

Recently, a study indicated that *Pseudomonas putida* increases the activity of soil urease, phosphatase and invertase, providing evidence that microbes can improve soil enzyme activity (Nosheen et al. 2018). Effective utilization of plant-growth-promoting bacteria (PGPB) is a key strategy in improving nutrient transformation and soil fertility. Inoculation with biofertilizers enhanced the activity of rhizosphere dehydrogenase, nitrogenase and alkaline phosphatase (Aseri et al. 2008). Roots of most plants are colonized by arbuscular mycorrhizal fungi (AMF) and these fungi acquire nutrients from soil, in particular P, and transfer them to host plants. In addition, AMF also transfer nitrogen to the host plants from decomposing litter, and AMF can change nitrogen cycling (Hodge and Fitter 2010; Nuccio et al. 2013; Smith and Read 2010). *Bacillus* RC01 as plant growth promoting bacteria (PGPB) significantly affected the content of both total mineral N and NO_3^--N of soil, and the content of soil-available phosphorus; however, PGPB did not affect soil pH and organic matter (Canbolat et al. 2006). It was demonstrated that nitrogen-fixing and phosphate-solubilizing bacteria significantly enhanced available N and P content in rhizosphere soil compared to the no-inoculation condition (Yu et al. 2012). In summary, AMF and PGPB keep the soil rich in nutrients via potassium and phosphate solubilization, nitrogen fixation, organic matter biodegradation and production of antibiotics in the soil (Sinha et al. 2010). However, we do not know whether the presence of an *Epichloë* endophyte in grasses affects soil nutrients, including possible different effects at different growth stages of host grasses. This contrasts with the sound knowledge of the role of AMF as an important regulator of ecological function.

The *Epichloë* endophytes form systemic associations, typically symptomless, with many cool-grasses, and which have important value in food chains of ecosystems. Over forty years of research have investigated the benefits of *Epichloë* endophytes for cool-season grasses. Many studies have centered on tall fescue (*Festuca arundinacea*) and perennial ryegrass (*Lolium perenne*), important grasses for pastures for livestock. Another grass that hosts an *Epichloë* endophyte is

Achnatherum inebrians, a grass that is increasingly common in the semi-arid/arid grasslands in China and this association has been extensively studied for some 15 years (Zhang et al. 2010; Zhang et al. 2012; Wang et al. 2018a, Wang et al. 2018b; Xia et al. 2016; Xia et al. 2018; Wang et al. 2019). These fungal endophytes/grass associations are a unique model system for the study of the relationships below and aboveground in ecosystems. *Epichloë* endophytes form host-specific, intercellular, mutualistic associations with host grasses with all tissues, apart from roots, being colonized (Christensen et al. 2008). These fungi are not found in nature apart from within host grasses. The endophyte is vertically transmitted in the seed of nearly all host grasses (Siegel et al. 1984). In view of the evidence that soil microbes can affect soil enzymes a question that arises is if the presence of these fungal endophytes affects soil enzymes? Further, what might be the ecological role of these endophytes from their influencing of the activity of enzymes and the nutrient availability of soil? Many ecological effects resulting from the presence of *Epichloë* endophytes in grasses are well documented, and an increasing number of studies are revealing the roles of *Epichloë* endophytes on microbial populations within soil. Perhaps the first study revealing the effect of an *Epichloë* endophyte on soil microbes was that endophyte-infection of tall fescue leads to a small differences of the soil microbial community, as determined with the fatty acid methyl ester method (Franzluebbers et al. 1999). This was followed by the finding of reduced mycorrhizal colonization of *E. occultans*-infected *L. multiflorum*; interestingly, the *Epichloë*-infected plants increased AM colonization in adjacent *Epichloë*-uninfected *L. multiflorum* plant. *Epichloë*-infected *Bromus setifolius* plants had significantly enhanced AMF hyphal length and branches compared to E− *B. setifolius* plants, because the root exudates were significantly different between E+ and E− plants; probably, *Epichloë* endophyte presence stimulated root exudates to cause the change of microbial communities (Novas et al. 2011; Omacini et al. 2006; Omacini et al. 2012). In contrast to the positive effects, it was reported that the presence of *E. coenophiala* in tall fescue did not clearly alter AMF colonization and AMF hypha length; in addition, *E. coenophiala* presence may not significantly shift nutrient cycling (Slaughter and McCulley 2016). Since then a number of studies have confirmed the ecological role of *Epichloë* endophyte. These include the *E. coenophiala* infected-tall fescue affects root-associated fungi, and the interaction of climate change and *E. coenophiala* presence influence symbioses of tall fescue, including arbuscular mycorrhizal fungi and dark septate endophyte colonization (Slaughter et al. 2018; Slaughter et al. 2019). Interestingly, the presence of *E. festucae* did not markedly influence the structure of culturable fungal endophyte in roots of *F. rubra* subsp. *pruinosa* compared to that of *E. festucae* uninfected-plants, probably, because some of the fungal species present were not isolated and identified (Pereira et al. 2019). *Epichloë* endophyte-infected tall fescue has been shown to significantly affect soil fungal communities, but roles of this fungal endophyte was less pronounced in bacterial communities. Further, it has also been shown that the presence of *E. coenophiala* did not influence total fungal biomass, but caused a change in the rhizosphere and the bulk soil fungal community composition (Rojas et al. 2016), and to cause a small differences of the soil microbial community as determined by use of the fatty acid methyl ester method (Franzluebbers et al. 1999). Interestingly, one study showed that *E. gansuensis*-infected *A. inebrians* increased rhizosphere soil nutrients content, including nitrate N, ammonium N, and available phosphorous, and both soil disturbance and *E. gansuensis* presence resulted in altering the root-associated fungi communities (Zhong et al. 2018). *E. coenophiala* clearly affected soil N content, but not non-symbiotic or symbiotic biological nitrogen fixation, and *E. coenophiala* also cause different impacts on nitrogen utilization and tall fescue's competitive ability. In addition, *E. coenophiala* improved the physical quality of soil due to influencing carbon and nitrogen cycling (Guo et al. 2016; Hosseini et al. 2017; Slaughter et al. 2016). However, very little is known about the ability of the endophyte to affect belowground soil properties, including enzyme activity and soil nutrients at the different growth stages of host grasses. This lack of knowledge provided the focus of this study of ecological impacts of *Epichloë* endophytes in field-growing plants.

The aims of this study are to determine (1) the effects of the *Epichloë* endophyte on the soil enzymes activity at the growth and senescence stages of *A. inebrians*, (2) the effects of the *Epichloë* endophyte on the soil nutrients at the growth and senescence stages of *A. inebrians*, (3) the effects of the interaction of *Epichloë*-infection and growth phase on the activity of soil enzymes and soil nutrient content.

Materials and methods

Experimental site description and soil sample collection

The experimental site, established at 2011, is located at Yuzhong campus of Lanzhou University, which is located at a latitude of 35°89′N, longitude of 104°39′E and altitude of 1653 m. In this study, the E+ (*A. inebrians* with *E. gansuensis*) and E− (*A. inebrians* without *E. gansuensis*) seeds originated from a single E+ seeds. To obtain E− plants for the study, E+ seeds from the single E+ plant were treated with a 100 times dilution of the fungicide thiophanate methyl for 2 h following which they were washed in sterile water (Li et al. 2016). To assess the effectiveness of the fungicide treatment seedlings grown from the treated fungicide treated seeds were examined for the presence of the characteristic intercellular hyphae in leaf sheaths that had been stained with aniline blue. Two areas of a field with either E+ or E− *A. inebrians* were established using the two seed lots originating from the single E+ plant. Representative plants of the trial areas were also assessed for the presence or absence of endophyte by examining leaf sheaths stained with aniline blue. 12 plots (1.05 m × 2.45 m) of E+ plants (2 lines × 6 rows) and 12 plots (1.05 m × 2.45 m) of E− plants (2 lines × 6 rows) were established; the distance between each E+ plant was 35 cm, and the distance between each E− plant was also 35 cm, the distance between E+ plants and E− plants was 2.5 m. 12 plots were one independent biological replicate, and there were three independent biological replicates in our study, including 36 plots for E+ and E− plants, respectively. During the six years after establishment, the trial area was subject to natural environmental conditions, and domestic and wild animals did not graze the areas. The mean annual rainfall was 400 mm year^{-1}, and the annual mean temperature was 11.6 °C at 2016.

In 2016, six years after establishment, the frequency of E+ plants were 100 % and 0% in the E+ and E− experimental field, respectively. The field soil was sampled in the growth stage (May 2016) and senescence stage of *A. inebrians* (August 2016). These dates correspond to the maximum growth stage and the time when plant growth is slowing and many senescent leaves are present, respectively. Three field soil samples were collected within 6-10 cm soil depth from the E+ and E− *A. inebrians* experimental fields, respectively, and the top 5 cm of the topsoil was removed. In brief, in every independent biological replicate, we collected four soil samples from the middle of the two lines in the second row, the third row, the fourth row and the fifth row, respectively, and these four individual soil samples were blended into a composite soil sample in every independent biological replicate. In addition, we choose different sites for the second collection soil samples in growth stage and the senescent stage, respectively, and the distance was 5cm between the growth stage site and the senescent stage site. The samples were transferred into dry, clean and sterile polythene bags with a sterile spatula. The soil samples were passed through a 2 mm sieve, and one part of the soil sample was air-dried and used for the analysis of soil nutrients. The other part of the sieved soil sample was stored at 4 °C for use for the analysis of soil enzymes activity.

Soil enzymes assay

Soil invertase activity and soil urease activity was determined with minor modification by Akhtar et al. (2018) and Zhang et al. (2011). In brief, the activity of soil invertase was assayed by addition of 5 g soil, 15 mL 8% glucose solution, 5 drops toluene and 5 mL 0.2 M phosphate buffer (pH 5.5) to a 50 ml volumetric flask, which was incubated for 24 h at 37 °C. After 24 h, the incubation mixture was filtered, and 1 mL filtered solution was treated with 3,5-dinitrylsalicylate (3 mL) in a 50 ml volumetric flask, and heated for 5 min. When the reaction liquid was cool, we measured it with an ultraviolet spectrophotometer at 508 nm. The soil urease activity was measured by addition of 5 g soil, 1 mL toluene, 10 mL 10% urea solution and 20 mL citrate buffer (pH 6.7) to a 50 ml volumetric flask, which was incubated at 37 °C for 24 h. The reaction mixture was filtered, and 1 mL filtered solution was reacted with 0.9% NaClO solution (3 mL) and sodium phenol solution (4 mL); 20 min after reaction, it was measured with an ultraviolet spectrophotometer at 578 nm. The activity of soil alkaline phosphatase was assayed with minor modification by the procedure of Tian et al. (2017). In brief, the activity of alkaline phosphatase was measured by using 5 g soil, 2 mL toluene, 20 mL 0.5% disodium phenyl phosphate and 10 mL borax buffer with pH 9.4 in a 50 ml volumetric flask, which was incubated at 37 °C for 24 h. After 24 h, the mixture was filtered, then the filtrate solution was reacted with 8% potassium ferrocyanide (0.5 mL) and 2% 4-aminoantipyrine; the activity was determined with an ultraviolet spectrophotometer at 510 nm.

Soil sample analyses

The soil samples were air dried, and passed through a 2 mm sieve, and the soil organic C (SOC) content was assayed (Nelson and Sommers 1982). Soil total nitrogen (TN) content, total phosphorus (TP) content, the soil ammonium N (NH_4^+, AN) and nitrate N (NO_3^-, NN) were determined (Zhao et al. 2014). Available soil phosphorus (AP) was quantified (Nelson and Sommers 1982).

Calculations and statistical analysis

Data analyses were carried out with SPSS version 17.0. Two-way ANOVA was used to determine the growth phase and endophyte on soil invertase activity, soil urease activity and soil alkaline phosphatase activity, SOC, TN and TP content, soil C:N, soil C:P, soil N:P, NN AN and AP. Significance difference between E+ and E− plant field soil for the significant parameters of growth phase × endophyte, and it was carried out at $P<0.05$ (independent T-tests). Non-metric multidimensional scaling (NMDS) analysis was performed with soil enzymes and soil nutrients by R (version 3.2.2), and NMDS with using the Bray-Curtis dissimilarity (Liu et al. 2018).

Results

Effects of *Epichloë gansuensis* and growth phase on soil invertase activity

Our results showed that the endophyte-infection (E) had a clear impact on the response of soil invertase activity ($P=0.002$, Table 1), but the growth phase (GP) did not significantly influence the soil invertase activity ($P=0.491$, Table 1), and the interaction of endophyte-infection and growth phase did not influence soil invertase activity (Table 1). Further, the infection of *E. gansuensis* increased soil invertase activity compared with the E− soil in the growth and senescence stages, with invertase activity of E+ soil being enhanced by 28.0 % and 9.5 % compared with the E− soil, respectively (Fig. 1a).

Effects of *Epichloë gansuensis* and growth phase on soil urease activity

Similarly, we found that the endophyte-infection (E) had a clear impact on the response of soil urease ($P=0.017$, Table 1), but the growth phase (GP) did not influence the soil urease activity ($P=0.312$, Table 1); further, the interaction of endophyte-infection and growth phase did not influence soil urease activity (Table 1). The soil urease activity showed differences between E+ and E− soil in the growth stage, with *E. gansuensis* enhancing the activity of soil urease compared with the E− soil, and E+ soil urease activity was enhanced by 10.4 % compared with E− soil (Fig. 1b).

Effects of *Epichloë gansuensis* and growth phase on soil alkaline phosphatase activity

The two-way ANOVA showed that the endophyte-infection (E) caused clear effect on the response of soil alkaline phosphatase ($P<0.001$, Table 1), and the growth phase (GP) had also significant differences in soil alkaline phosphatase ($P<0.001$, Table 1). However, the interaction of endophyte-infection and growth phase did not influence soil alkaline phosphatase activity (Table 1). We found that the infection of *E. gansuensis* enhanced soil alkaline phosphatase activity compared with E− soil, being enhanced by 11.2 % and 29.6 % compared with E− soil in the growth and senescence stages of *A. inebrians*, respectively (Fig. 1c)

Soil organic C, total N and total P content and stoichiometry of E+ and E− soil nutrients in the growth and senescence stages

The endophyte-infection (E) had a clear role on the response of SOC ($P<0.001$, Table 1) and TN ($P<0.001$, Table 1), but the E did not influence the TP content ($P=0.281$, Table 1; Fig. 2c). The growth phase (GP) had also pronounced differences in TN ($P<0.001$, Table 1), but GP did not impact the content of SOC ($P=0.666$, Table 1) and TP ($P=0.107$, Table 1). E × GP interaction had clear differences in TN ($P=0.01$, Table 1), but the interaction of E × GP had no difference in the content of SOC ($P=0.575$, Table 1) and TP ($P=0.513$, Table 1). Furthermore, we found that E+ soil organic C was higher compared with the E− soil, and the E+ soil organic C content was enhanced by 26.4 % and 20.0 % compared with E− soil in the growth and

Table 1 Results of two-way ANOVA for the effects of endophyte (E) and growth phase (GP) on soil invertase activity, soil urease activity, soil alkaline phosphatase activity, soil organic carbon (SOC) content, soil total nitrogen (TN) content and soil total phosphorus (TP) content

Treatments	df	Invertase		Urease		Alkaline phosphatase		SOC		TN		TP	
		F-value	*P*	F-value	*P*	F-value	*P*	F-value	*P*	F-value	*P*	F-value	*P*
E	1	19.13	**.002**	8.97	**.017**	48.65	**<0.001**	26.30	**<0.001**	79.64	**<0.001**	1.34	0.281
GP	1	0.52	.491	1.16	.312	148.9	**<0.001**	0.20	.666	115.89	**<0.001**	3.3	0.107
E * GP	1	3.89	.084	2.91	.126	4.06	0.079	.34	.575	11.49	**.010**	0.47	0.513

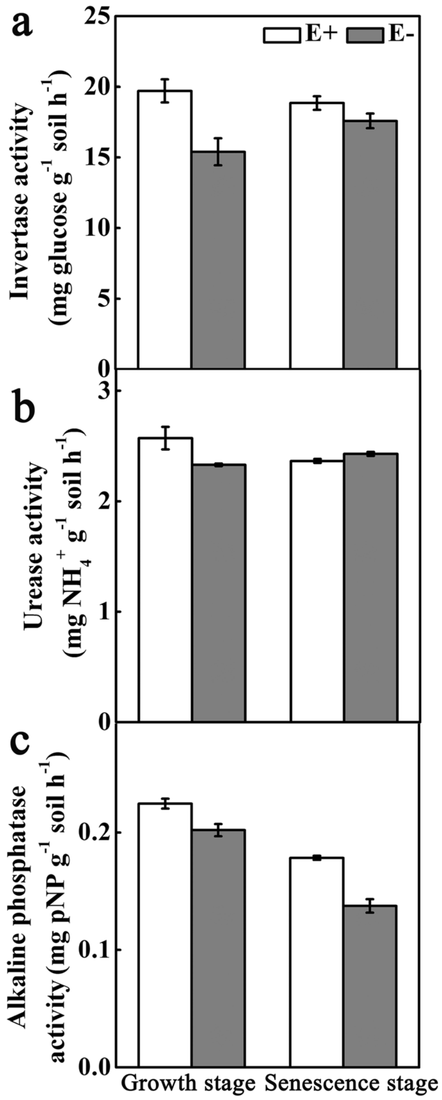

Fig. 1. Effect of *E. gansuensis* on soil invertase activity (a), soil urease activity (b) and soil alkaline phosphatase activity (c) at the growth and senescence stages. Data are mean of three replicates, and the bars are standard errors.

senescence stages, respectively (Fig. 2a). Similarly, the content of soil total N (TN) showed a similar pattern to the content of soil organic C, being higher in E+ soil than in E− soil, with the TN content in E+ soil being increased by 20.6 % and 7.5 % compared with E− soil in growth and senescence stages, respectively (Fig. 2b).

The results of two-way ANOVA showed that E caused a marked effect on the response of C:P (P<0.001, Table 2) and N:P (P<0.001, Table 2), but E did not influence the C:N (P=0.169, Table 2). GP had also clear differences in C:N (P=0.03, Table 2) and N:P (P=0.01, Table 2), but GP did not influence the C:P (P=0.505, Table 2). The interaction of E × GP had no difference in C:N, C:P and N:P (Table 2). Interestingly, there was a higher C:N in E+ soil compared to E− soil in the senescence stage (Fig. 3a), And the soil C:N in E+ soil was enhanced by 11.6 % compared with the E− soil in the senescence stage (Fig. 3a). Our results showed that the soil C:P ratio in E+ soil was increased by 32.1 % and 21.5 % compared with E− soil during the growth and senescence stages, respectively (Fig. 3b). However, a higher N:P in E+ soil was seen when compared to the E− soil in the growth stage, being increased by 26.5 % (Fig. 3c).

The content of soil nitrate N (NO_3^-, NN), ammonium N (NH_4^+, AN) and soil available phosphorus (AP)

The results of two-way ANOVA showed that E caused a clear effect on the response of soil ammonium N (AN) (P<0.001, Table 3) and soil available phosphorus (AP) (P<0.001, Table 3), but soil nitrate N (NN) was not influenced by E (P=0.631, Table 3; Fig. 4a). GP also had marked differences in NN (P<0.001, Table 3) and AP (P=0.027, Table 3), while AN was significantly influenced by GP (P=0.441, Table 3). E × GP interaction had a significant effect on the response of AN (P=0.012, Table 3), E × GP interaction had no

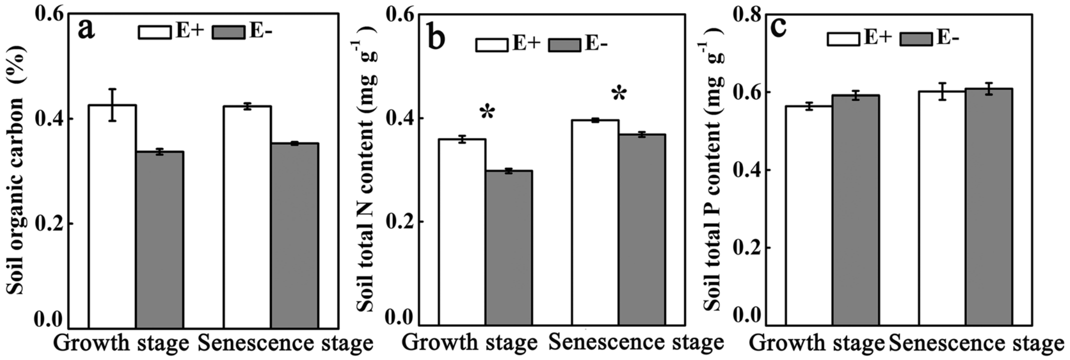

Fig. 2. Effect of *E. gansuensis* on soil organic C (a), soil total N (b) and soil total P contents (c) in the growth and senescence stages. Data are three replicates mean, and the bars indicate standard errors. Asterisk stand for significant difference at $P<0.05$ (independent T-tests) between E+ and E− soil nutrients.

difference in NN (*P*=0.479, Table 3) and AP (*P*=0.128, Table 3). Further, our results showed that the AN content in E+ soil was enhanced by 53.1 % compared with that of E− soil in the growth stage (Fig. 4b). The content of AP in the growth and senescence stages in E+ soil was increased by 218.5 % and 183.5 % compared with E− soil, respectively (Fig. 4c).

The NMDS analysis of soil enzymes and soil nutrients

The NMDS analysis showed that E+ and E− are significantly separated for each sample and individual sampling in the growth and senescence stages, respectively, and the growth and senescence stages are clearly separated in E+ soil and E− soil, respectively; and the value of stress was 0.0347, which was shown to be a good fit for NMDS analysis (Fig. 5, Table 4). The effect of endophyte-infection could explain 72.8% of soil enzymes and nutrients variation (Adonis, *P*=0.001) (Table 4). Meanwhile, the growth phase considerably affected soil properties, accounting for 10.6% of soil enzymes and nutrients variation (Adonis, *P*=0.045) (Table 4).

Table 2 Results of two-way ANOVA for the effects of endophyte (E) and growth phase (GP) on soil C:N, soil C:P and soil N:P

Treatments	df	C:N		C:P		N:P	
		F-value	*P*	F-value	*P*	F-value	*P*
E	1	2.280	.169	33.974	**<0.001**	25.492	**<0.001**
GP	1	6.905	**.030**	0.488	.505	11.407	**0.01**
E × GP	1	.243	.635	1.227	.300	4.718	0.062

Discussion

In the present study, soil enzymes and the soil nutrients were affected by the presence in *A. inebrians* plants of the mutualistic fungal endophyte *E. gansuensis*, and by the plant growth phase. There is a need to integrate the soil enzymes into our understanding of ecosystem interactions between *A. inebrians* and *E. gansuensis*. This research provides insights into how *A. inebrians* symbiotic with *E. gansuensis* affected three enzymes and nutrients present in soil in the growth stage during the peak growth season, and later in the senescence stage, when many leaves were becoming senescent.

Soil enzyme activity is an important indicator for the ecosystem sustainability and health (Cao et al. 2015), and soil enzymes play also central role in maintaining the soil fertility. The results of the present study revealed that *E. gansuensis* increased the soil invertase activity and SOC content in growth and senescence stages of *A. inebrians*. This finding is consistent with previous reports about soil invertase and how its activity is linked to microorganisms, and the study found that *Pseudomonas putida* increased soil invertase activity; in addition, fungi, bacteria and yeast secreted invertase into the soil medium, which also lead to soil invertase activity increase (Nosheen et al. 2018; Shah et al. 2013). It has been found that the activity of soil invertase, soil urease and soil alkaline phosphatase were positively related with SOC and microbial biomass, and this suggests that these fractions contain substrates that induce synthesis of these enzymes synthesis (Bastida et al. 2006; Zhang et al. 2011; Zhang et al. 2020). Soil invertase is a key indicator of soil fertility and varies with the different land types, and it is involved in the decomposition/

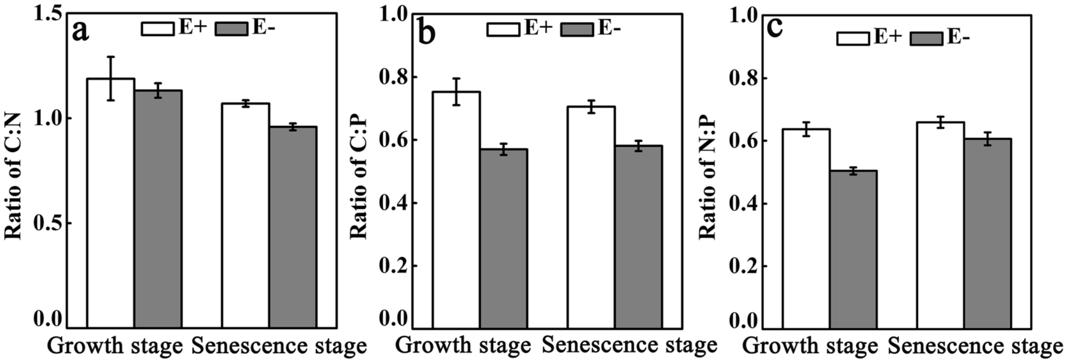

Fig. 3. Effect of *E. gansuensis* on soil C:N (a), C:P (b) and N:P ratio (c) at the growth and senescence stages. Data are three replicates mean, and the bars indicate standard errors.

transformation of soil organic matter. Research showed that soil invertase had a positive relationship with soil organic matter and bacterial diversity (Gong et al. 2019; Wei et al. 2015). Recently, it has been reported that *E. gansuensis* increased Chao1 richness index and Shannon diversity of the rhizosphere soil bacterial community of *A. inebrians* (Ju et al. 2020). Therefore, this increase in soil invertase activity may be due to *E. gansuensis* enhancing bacterial diversity. A previous study showed the straw mulch treatment increased the activity of soil invertase, soil urease and soil alkaline phosphatase through increased microbial population and microbial biomass C or N, which provided SOC that was used as a substrate for soil enzymes (Martens et al. 1992). Therefore, the findings of our study may have been because the microbial diversity and SOC were increased by the presence of *E. gansuensis* in *A. inebrians*.

Our results have also shown that *E. gansuensis* increased soil urease activity at the growth stage. Soil urease is involved in the hydrolysis of urea, and enhances nitrogen fertilizer utilization rate (Maithani et al. 2017). It has been found that AMF and bacteria like *Pseudomonas putida*, *Azospiriullum brasilense* and *Methylobacterium oryzae* increased soil urease activity (Madhaiyan et al. 2010; Nosheen et al. 2018; Xun et al. 2014). Importantly, the activity of soil urease was increased with enhancement in soil microbial populations, and the soil urease activity was positively correlated with soil bacterial diversity (Gong et al. 2019). As previously stated, Ju et al. (2020) reported that *E. gansuensis* increased bacterial community diversity of the rhizosphere soil in *A. inebrians*. The increased soil urease activity at the growth stage of *E. gansuensis* infected *A. inebrians* was found in our study, which may be due to the presence of *E. gansuensis* increasing microbial diversity. This implication is consistent with previous reports, that with the increase of microbial populations, soil urease activity also increased, and this leads to improvement of the soil fertility (Bansal et al. 2014).

Another finding of our study was that the presence of the endophyte in *A. inebrians* increased soil phosphatase activity. Soil phosphatase is involved in organic phosphorus mineralization (Huang et al. 2011). It has been found that the inoculation with bacteria like *Bacillus cereus* Pb25, *Pseudomonas putida* and *Pseudomonas* increased the activity of soil phosphatase and rhizosphere phosphatase, respectively, and through rhizosphere organic phosphate mineralization into available

Table 3 Results of two-way ANOVA for the effects of endophyte (E) and growth phase (GP) on soil nitrate N (NN) content, soil ammonium N (AN) content and soil available phosphorus (AP) content

Treatments	df	NN		AN		AP	
		F-value	*P*	F-value	*P*	F-value	*P*
E	1	.249	.631	35.258	**<0.001**	63.045	**<0.001**
GP	1	61.125	**<0.001**	0.657	.441	7.341	**0.027**
E × GP	1	.552	.479	10.285	**.012**	2.886	0.128

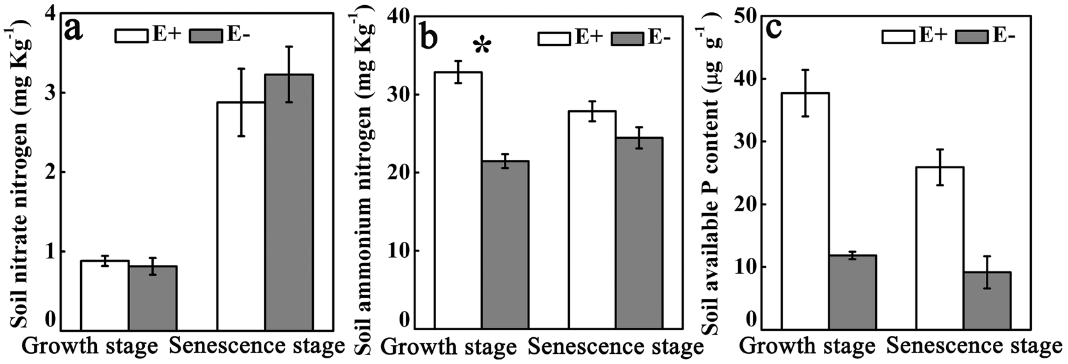

Fig. 4. Effect of *E. gansuensis* on soil nitrate nitrogen (a), ammonium nitrogen (b), available phosphorus (c) in the growth and senescence stages. Data are three replicates mean, and the bars are standard errors. Asterisk stand for significant difference at $P<0.05$ (independent T-tests) between E+ and E− soil available nutrients.

phosphate (Hussain et al. 2013; Islam et al. 2016; Nosheen et al. 2018). The activity of soil phosphatase has been found to be positively correlated with fungi diversity (Gong et al. 2019). On the contrary, the process of increased phosphatase activity in soil may not be a consistent outcome of inoculation with microbes as inoculation with *Pseudomonas* and *Trichoderma* significantly decreased soil phosphatase activity (Mar Vázquez et al. 2000). It is probable that soil phosphatase activity was related to soil nutrients and soil microbial community. Zhong et al. (2019) reported that the *Epichloë* endophyte in *A. inebrians* increased the diversity of root-associated AM fungal under drought conditions; thus, enhanced phosphatase activity in the rhizosphere probably is associated in part with the presence of AMF. The finding of increased phosphatase in our study may have been because the microbial diversity was increased by the presence of *E. gansuensis*in in *A. inebrians* plants.

Our research showed that the activity of these three enzymes independently differed in the growth and senescence stages. A previous study demonstrated that the activity of soil invertase and soil urease were positively related to soil organic carbon, soil total nitrogen, soil total phosphorus and available phosphorus (Gong et al. 2019), which are consistent with our results. *E. gansuensis* increased soil invertase activity, soil urease activity, soil phosphatase activity, soil organ carbon, soil total nitrogen, AN and AP content.

In our study, soil under *A. inebrians* with *E. gansuensis* had a greater soil total N and soil organic C content than without *E. gansuensis* infection in the growth and senescence stages. This finding is in agreement with the presence of *E. coenophila* in tall fescue where a high ratio of *Epichloë* -infection gave rise to a greater content of soil N and soil organic C than with a low *E. coenophila* -infection ratio, and the presence of *Epichloë* in tall fescue influenced soil C and N cycling (Franzluebbers et al. 1999; Franzluebbers and Stuedemann 2002; Guo et al. 2016; Iqbal et al. 2012), this effect was probably caused via the *Epichloë* influencing the diversity of soil microbes and soil

Fig. 5. Non-metric multidimensional scaling analysis (Bray-Curtis dissimilarity) for endophyte-infection and growth phase with soil enzymes and soil nutrients.

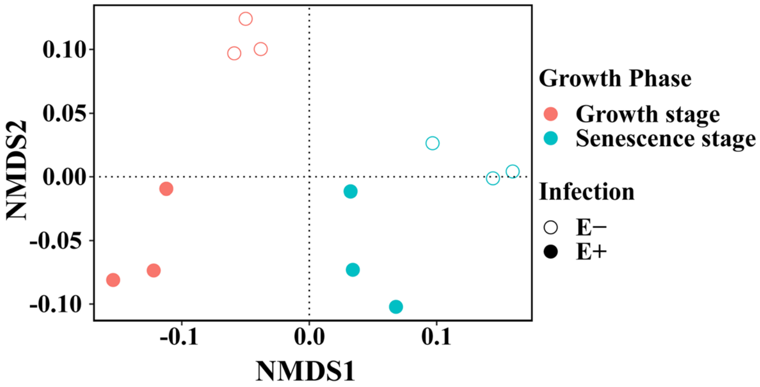

Table 4 Results of Adoins analysis for the effects of growth phase and endophyte infection on soil enzymes activity and soil nutrients; and the value of stress for NMDS analysis with soil enzymes activity and soil nutrients

	Growth Phase		Endophyte Infection	
	R^2	*P*	R^2	*P*
Adoins	0.106	**0.001**	0.728	**0.045**
Stress	**0.0347**			

enzymes. However, there was a report that *Epichloë*-infection of meadow fescue (*F. pratense*) did not affect soil N and C content, and soil NO_3^- and NH_4^+ content were differences between where E+ and E− meadow fescue is growing (Mikola et al. 2016). Possibly this indicates that different grass-*Epichloë* associations have different effects on soil properties. Interestingly, one study showed that the inoculation with AMF + PGPR significantly upregulated nitrate and ammonium transporter genes of durum wheat in the absence of organic N (Saia et al. 2015). Therefore, *E. gansuensis* maybe influences the expression of nitrate and ammonium transporter genes of *A. inebrians*, which may enable host plants to adapt to a poor soil environment. One viewpoint is that the differences of alkaloid amount and type may be a factor in the differences of soil N and C content in endophyte-free and endophyte-infected tall fescue stands (Lemons et al. 2005). *E. coenophiala* NTE19 had significantly greater soil extraradical hyphae compared to *E. coenophiala*-free tall fescue plants, and AMF are associated with improving soil C sequestration and physical characteristics (Slaughter et al. 2019); therefore, *Epichloë* may influence the AMF hyphae to improve soil quality. It has been found that inoculation of PGPB, phosphate-solubilizing bacteria or nitrogen-fixing bacteria like *B. cereus* Pb25, *Bacillus megaterium* and *Arthrobacter pascens* enhanced soil AP, N and total organic carbon compared to soil not inoculated with these bacterial strains (Islam et al. 2016; Kpomblekou and Tabatabal, 1994; Yu et al. 2012). One study has demonstrated that an increase in the soil microbes diversity was found in response to fertilizer management, and this response was closely correlated to the soil N:P and C:N ratios, and significantly increased N:P ratios in the soil that would enhance microbial diversity and biomass, and soil C:N and C:P were positively related with soil bacterial diversity, and soil N:P were positively correlated with soil fungal diversity (Liu et al. 2020). Our research also showed that *E. gansuensis* infection in *A. inebrians* promoted the soil C:P in both the growth and senescence stages, and soil C:N ratio in *A. inebrians* in the senescence stage and the soil N:P ratio in the growth stage. Further, our results also demonstrated that *E. gansuensis* infection in *A. inebrians* not only promotes soil AN content accumulation in associated soils in the *A. inebrians* growth stage, but also promotes soil AP content accumulation in soils in both the growth and senescence stages. Therefore, *E. gansuensis* endophyte probably influenced the P-solubilizing activity of soil microbes, which increased soil AP content.

One study has demonstrated that bacterial diversity was positively correlated with SOC, TN, TP, NN and AP (Gong et al. 2019). Ju et al. (2020) demonstrated that *E. gansuensis* in *A. inebrians* increased bacterial diversity of the rhizosphere soil, which maybe explains how this *Epichloë* species improved soil nutrients. It has been found that inoculation of *Bacillus* RC01, or *Phomopsis* sp. endophyte or *Phomopsis liquidambari* could improve plant growth through increasing the content of soil AP and soil NN, the activity of soil carbon metabolic enzyme, or soil N cycling (Canbolat et al. 2006; Chen et al. 2010; Yang et al. 2015). Similarly, the presence of *E. occultans* increased *L. multiflorum* biomass, and N and P accumulation, which led to improve plant growth (Omacini et al. 2006), and this phenomenon may be caused by the presence of the *Epichloë* increasing soil available nutrients; for example, NN, AN and AP. Therefore, *E. gansuensis* probably improved soil enzymes activity through influencing soil microbial diversity, and soil enzymes are involved in the decomposition of soil nutrients, which leads to improve soil fertility. It seems very likely that the changes in soil enzyme activity and soil nutrient content resulting from *E. gansuensis*-infection in *A. inebrians* will result in improved plant growth. Interestingly, the presence of *E. festucae* did not significantly affect the core microbiota structure in the roots of *F. rubra* subsp. *Pruinosa* (FRP), when compared to that of plants without this endophyte, but the core mycobiome of FRP had an important role in host grass adaptation to NaCl stress (Pereira et al. 2019).

These results implied that *E. gansuensis* has important ecological functions resulting from changes occurring in the soil, affecting soil fertility, presumably from changes to the composition of soil bacteria and maybe other microorganisms. These responses might reflect differences in soil nutrients that could interact with

Epichloë-host grass symbiosis to change soil nutrient absorption. For example, it suggests that the presence of the *Epichloë* endophyte may improve tall fescue adaptation to low P conditions through modulating nutrient uptake (Malinowski et al. 1999; Malinowski et al. 2000; Rahman and Saiga 2005; Guo et al. 2016). Our research showed that growth stage and senescence stage have different influences on the different soil nutrient parameters. Interestingly, our results indicated that the effects of *E. gansuensis* on soil AN content were different in the growth and senescence stages. *Epichloë* species colonize all plant tissues except roots, and so why do they affect soil properties? These *Epichloë* endophyte may affect the physiology of the host, thereby affecting root exudation, influencing the rhizosphere microorganism structure, affecting the soil enzymes and soil nutrients. The improvement in soil properties conferred by *E. gansuensis* would provide a competitive advantage over *Epichloë*-free *A. inebrians* plants and may be another reason why nearly all *A. inebrians* grasses are *Epichloë*-infected in the nutrient poor soils of the vast grasslands of northwest China.

This study examined how the interaction of *A. inebrians* and *E. gansuensis* affected soil enzymes activity and soil nutrients content in the growth stage when peak plant growth is occurring and in the senescence stage when many leaves are senescing. We found that the *E. gansuensis* increases soil invertase and alkaline phosphatase activity in both the growth and senescence stages, and *E. gansuensis* enhanced urease activity in the growth stage. Further, our results demonstrated that the *E. gansuensis* improves soil nutrition; for example, *E. gansuensis* increases SOC, soil TN, soil AN and soil AP. The presence of *E. gansuensis* was a key factor to impact soil enzymes activity and soil nutrients. Overall, in this study, the *E. gansuensis* increased SOC and soil TN, which indicated that this well-known mutualistic grass-*Epichloë* symbiosis might have potential as a source of soil C and N sequestration. Further, enhanced soil nutrients will improve the yield of herbage and provides another reason why the presence of *Epichloë* endophytes in grasses could enhance the forage economic value in sown pastures and natural rangelands.

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Compliance with ethical standards

Conflict of interests The authors declare that they have no competing interests

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