Pak. J. Agri. Sci., Vol. 56(1), 171-178; 2019
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI:10.21162/PAKJAS/19.5605
http://www.pakjas.com.pk

CORRELATION ANALYSIS OF NUTRIENTS, ENZYMES, AND MICROBIAL BIOMASS IN SOILS WITH PHENOLICS OF *Artemisia annua* L.

Shi-Qiong Luo[1], Cheng Zhao[1], Zhan-Nan Yang[2,*], Sheng-Juan Di[2], Zhu Zheng[2] and Juan Hu[2]

[1]School of Life Science Guizhou Normal University, Guiyang, Guizhou Provence, 550001, P. R. China; [2]Key Laboratory for Information System of Mountainous Area and Protection of Ecological Environment of Guizhou Province, Guizhou Normal University, Guiyang, Guizhou Provence, 550001, P. R. China
[*]Corresponding author's e-mail: yangzhannan@163.com

A correlation analysis of the nutrients, enzymes, and microbial biomass in *Artemisia annua* L. rhizosphere soil with *A. annua* phenolics was performed to assess whether the properties of *A. annua* rhizosphere soil could affect *A. annua* phenolics. The concentrations of soil nutrients, enzyme activities, microbial biomass C and N, total polyphenols, scopoletin, chrysosplenol-D, and chrysosplenetin significantly varied at different sampling sites. The total polyphenols were significantly positively correlated with available K, invertase, and microbial biomass C, but negatively correlated with organic matter, urease, and phosphatase. Significant positive correlation was observed between scopoletin and organic matter, available P, phosphatase, and microbial biomass C and C/N ratio. Chrysosplenol-D was significantly positively related to available K and invertase, and significantly negatively related to phosphatase. Chrysosplenetin was significantly positively related to microbial biomass C, and negatively related to available N. These findings suggested that transformation of soil N and P is promoted by *A. annua* through activation of rhizosphere enzymes and microorganisms. This is advantageous for the adaptation of *A. annua* to a wide range of soil environments and influences the metabolic accumulation of *A. annua* phenolics.
Keywords: *Artemisia annua* L., microbial biomass, soil nutrients, metabolic accumulation, anti-malarial drug

INTRODUCTION

Artemisia annua L., an annual herb, has been used in traditional Chinese Medicine (TCM) for 2000 years due to its various medicinal properties (Chukwurah *et al.*, 2014; Chinese Pharmacopoeia, 2015). For instance, the main chemical component, artemisinin, isolated from *A. annua*, is the first-line anti-malarial drug recommended by the World Health Organization, and *A. annua* phenolics (total polyphenols, scopoletin, chrysosplenol-D, and chrysosplenetin) have been confirmed to synergize the potential effects of artemisinin against malaria and cancer (Jorge *et al.*, 2010). Nevertheless, the concentration of the effective components in *A. annua* (e.g. phenolics, artemisinin, *etc*.) is often ambiguous because of the influence of diverse growth environments. In recent decades, numerous studies have focused on artificial synthesis of artemisinin and on confirming the synergistic action of phenolics and artemisinin against malaria and cancer (Jorge *et al.*, 2010). However, as artificial synthesis of artemisinin and other components of *A. annua* is limited by complex and expensive procedures, these components are still isolated from *A. annua*. Moreover, the effect of environmental factors on the metabolic accumulation of *A. annua* phenolics that act in synergy with artemisinin against malaria and cancer is unclear and needs to be investigated. Thus, in the present study, we analyzed the correlation between nutrients, enzymes, and microbial biomass of *A. annua* soil environment and *A. annua* phenolics, and discussed whether the properties of *A. annua* soil environment could affect metabolic accumulation of phenolics in *A. annua*.

The metabolic accumulation of *A. annua* phenolics is closely related to complex climatic and soil conditions (Peng *et al.*, 2008; Luo *et al.*, 2014; Wang *et al.*, 2014). *A. annua* activates and utilizes soil nutrients (Kuklová *et al.*, 2015) and soil microorganisms that are involved in organic matter decomposition and transformation of nutrients (N, P, K, etc.), thus improving the biological availabilities of soil nutrients (Alina, 2011; Hu *et al.*, 2016). At the same time, the enzymes secreted by the microorganisms in the soil and roots of *A. annua* are also involved in the transformation and utilization of soil nutrients (Tischera *et al.*, 2015). It has been reported that artemisinic acid and artemisinin of *A. annua* are not only involved in *A. annua* growth, but also affect the nutrients and microorganisms in soil (Luo *et al.*, 2014; Awan *et al.*, 2015). Moreover, fertilization has also been found to influence the phenolic components of *A. annua* (Luo *et al.*, 2013). Thus, to investigate *A. annua* phenolics and soil properties, the correlations between *A. annua* phenolics and *A. annua* soil environment must be determined.

The present study provides evidence-based data to answer the key question about the effects of soil factors on the metabolic accumulation of phenolics in *A. annua*, which could adapt to a wide range of environments. Although this study had

specifically focused on *A. annua*, the methods employed can possibly be applied to other herbal plants to improve their quality.

MATERIALS AND METHODS

Experimental materials: Soil samples from the rhizosphere of *A. annua* were collected during the squaring stage from different sites from August to September 2014. Samples from the roots of nine *A. annua* plants were pooled and labeled as a replicate. Three replicates were acquired from each site. The rhizosphere soil was obtained by shaking off the soil on the roots and collecting the soil adhered to the root surface. Furthermore, at each site, three sampling points were chosen, and nine plants were collected from each sampling point. Each soil sample was collected in a sterile plastic bag, rapidly transported to the laboratory, and divided into two parts: one part was air-dried and used for the analysis of physical and chemical properties as well as enzyme activities and the other part was stored in a freezer at 4°C for the investigation of biomass C of soil microorganisms. The leaves and buds of the collected plants were respectively air-dried in a room and then ground into a powder for the analysis of *A. annua* phenolics. The information on sampling sites, geographic position, and soil types is presented in Table 1. Scopoletin, chrysosplenol-D, and chrysosplenetin were purchased from Sigma (USA). Acetonitrile and methanol (HPLC) were purchased from MERCK, Inc. (Germany). Formic acid was purchased from TianJin Chemical Reagents Development Center (TianJin, China). Ultrapure water (18.2 M) was prepared using Sartorius Arium 611UF water purification system (Sartorius, Germany). Caffeic acid was purchased from Shanghai Haling Biological Technology Company (China). Folin-Cioncalteau's phenol reagent was purchased from Labest Company (USA). All the reagents were of analytical grade.

***Analysis of total polyphenols*:** The total polyphenols were extracted from the *A. annua* samples using the method developed by Jessing *et al.* (2013) with minor modification. Briefly, 0.2 g of the leaves powder was added to 25 mL of methanol in a conical flask and sealed with a stopper and rubberized fabric. The flasks were incubated at 25°C on a rotary shaker at 141 rpm for 24 h. Subsequently, the content of the flask was filtered and the filtered liquor extract was used for the analysis of total polyphenols. The concentrations of total polyphenols were determined according to the method proposed by Martínez-Blanco *et al.* (2011) with slight modification. In brief, 0.5 mL of the extract was added to 3 mL of water and 0.1 mL of Folin-Cioncalteau's phenol reagent. After 5 min, 2.5 mL of 20% Na_2CO_3 and 3.9 mL of water were added to the mixture and mixed well. After 2 h, the color developed was measured at 725 nm using a spectrophotometer was purchased from Jiangsu Technology Company (China). The concentrations of total polyphenols were evaluated as caffeic acid equivalents with caffeic acid calibration curve.

***High-performance liquid chromatography analysis of scopoletin, chrysosplenol-D and chrysosplenetin*:** The pulverized samples (≈ 0.1 g) were accurately weighed and added into a 150-mL conical flask and sonicated with methanol (25 mL) for three times at 40°C (30 min each). The extracts obtained were centrifuged and concentrated to about 20 mL at ≈ 50°C by using rotary evaporators (R-210, BUCHI, Switzerland). Subsequently, the concentrated extracts were diluted to 25 mL with methanol and filtered through a 0.45-µm membrane filter. Then, the extracts were subjected to high-performance liquid chromatography (HPLC) analysis using a HPLC system LC-20AT series (Shimadzu, Japan). All chromatographic separations were performed on a Waters PAH C_{18}, 5 µm, 4.6 × 250 mm column (part No. 186001265). A linear gradient elution was performed using eluent A (methanol:acetonitrile, 5:11 (v/v)) and eluent B (formic acid (0.1%, m/v)) as follows: 5% A (0–5 min), 5%–16% A (5–8 min), 16%–24% A (8–30 min), 24%–32% A (30–47 min), 32%–64% A (47–68 min), 64% A (68–75 min), 64%–100% A (75–78 min), 100% A (78–88 min), 100%–5% A (88–90 min), and 5% A (90–95 min). The flow rate program was as follows: 1.4 mL/min (0–5 min), 1.4–0.6 mL/min (5–10 min), 0.6–0.8 mL/min (10–48 min), 0.8–1.4 mL/min (48–50 min),

Table 1. The information on sampling sites, geographic position, and soil types.

Site codes	Site names	Geographic positions	Elevations (m)	Soil types	>2mm gravels Content (%)
KL1	Shangxinzhai, Kaili	26°31′45″N, 107°49′33″E	632	Calcareous yellow soil	80.23
KL2	Xiaxinzhai, Kaili	26°31′27″N, 107°50′25″E	656	Calcareous yellow soil	61.34
KL3	Wantang, Kaili	26°49′63″N, 107°79′09″E	642	Quaternary Period yellow soil	12.75
GY1	Lijiazhuang, Guiyang	26°30′22″N, 106°39′17″E	1 135	Quaternary Period yellow soil	14.67
GY2	Baishacun, Guiyang	26°30′24″N, 106°38′56″E	1 159	Calcareous yellow soil	32.53
GY3	Baishaocun, Guiyang	26°29′54″N, 106°38′15″E	1 157	Calcareous yellow soil	59.85
QX1	Jiefangcun, Hongshuizheng, Qianxi	27°07′02″N, 105°98′98″E	1 262	Gravelly calcareous soil	82.45
QX2	Shibaocun, Supuzheng, Qianxi	26°99′98″N, 106°35′13″E	1 344	Calcareous yellow soil	8.27
QX3	Xuetangcun, Supuzheng, Qianxi	26°99′01″N, 106°34′72″E	1 267	Quaternary Period yellow soil	7.34

and 1.4 mL/min (50–95 min). The detection wavelength was set at 345 nm and the column temperature was maintained at 35°C.

***Determination of soil nutrient content and pH*:** The nutrient content and pH of the *A. annua* soil samples were analyzed according to the methods described elsewhere (Bao, 2005; Mehdi *et al*., 2018). The pH of the soil samples was measured using a pH meter (Saichang Scientific Instrument Company, China). Potassium dichromate oxidation heating method was employed to determine the soil organic matter, and Kjeldahl nitrogen determination method was applied to test the total N concentration in the soil samples. The total P concentrations in the soil samples were determined by Mo-Sb colorimetric method. In brief, the samples were digested with H_2NO_3-HF and transferred to a volumetric flask at a final volume of 100 mL and subjected to Mo-Sb colorimetric method. The total K concentrations in the soil samples were detected by flame photometry. The soil available P was extracted using 0.05 mol/L $NaHCO_3$ and measured by the Olsen method, the soil available K was extracted using 1 mol/L CH_3COONH_4 and determined by flame photometry, and the soil available N was determined by alkali solution diffusion (Zhang *et al.*, 2014).

***Analysis of soil enzyme activities and microbial biomass*:** Urease activity was measured by phenol-hypochlorite colorimetric method as follows: the soil sample was incubated with buffered 10% urea at 37°C for 24 h, and urease activity was evaluated as the amount of NH_4^+-N (in mg) per gram of soil after 24 h (mg NH_4^+-N/g/24h). Invertase activity was determined by 3,5-dinitrosalicylic acid method. Briefly, the soil sample was incubated with buffered 8% sucrose at 37°C for 24 h, and invertase activity was evaluated as the amount of glucose (in mg) per gram of soil after 24 h (mg glucose/g/24h). Phosphatase activity was analyzed using 2,6-dibromoquinone-4- chloroimide colorimetric method as follows: the soil sample was incubated with buffered 0.5% disodium phenyl phosphate hydrate, and phosphatase activity was evaluated as the amount of phenol (in mg) per gram of soil after 24 h (mg phenol/g/24h) (Guan, 1986). To measure the microbial biomass C in the soil, oxidation-reduction method was employed after the fresh soil was fumigated with trichloromethane, whereas indigo colorimetric method was applied to determine the microbial biomass N in the soil (Yao and Huang, 2006).

***Statistical analysis*:** All the soil samples were randomly collected in triplicates. Excel 2003 was applied to analyze the basic data of the experiment and construct graphs. SPSS 18.0 was used for the analysis of correlation, ANOVA, and LSD significant difference ($p < 0.05$).

RESULTS AND DISCUSSION

***Phenolic concentrations in A. annua leaves*:** Although *A. annua* phenolics and artemisinin exhibit a synergistic effect against malaria, the metabolic accumulation of *A. annua* phenolics varied with respect to different growth environments at different sites (Brisibe *et al.*, 2009; Jorge *et al.*, 2010). As shown in Table 2, the concentration of total polyphenols varied from 2.46 to 11.51 g kg^{-1} and was the highest in QX2 sample and lowest in GY3 sample. The mean concentration of total polyphenols in QX sample (8.28 g kg^{-1}) was higher than that in GY and KL samples (3.06 and 4.36 g kg^{-1}, respectively). Furthermore, although scopoletin concentrations in the KL1, KL2, and GY2 samples were not significantly different, they were significantly higher than those noted in other samples. The chrysosplenol-D content was the highest in QX2 sample (1.00 g kg^{-1}), followed by GY1 and QX1 samples, and was the lowest in GY3 sample. Moreover, the mean chrysosplenol-D concentration in QX sample (0.924 g kg^{-1}) was significantly higher than that in samples from other two regions (KL, 0.651 g kg^{-1}; GY, 0.647 g kg^{-1}). Although the mean chrysosplenetin concentrations among the three regions (KL, 0.750 g kg^{-1}; GY, 0.722 g kg^{-1}; QX, 0.697 g kg^{-1}) did not show any significant difference, they were significantly diverse at different sites, with the highest chrysosplenetin concentration noted in GY1. These results indicated that the concentrations of total polyphenols, scopoletin, chrysosplenol-D, and chrysosplenetin in *A. annua*

Table 2. The concentrations of phenolics from *A. annua*.

Site codes	Total polyphenols (g kg^{-1})	Scopoletin (g kg^{-1})	Chrysosplenpl-D (g kg^{-1})	Chrysosplenetin (g kg^{-1})
KL1	3.57±0.30d	0.92±0.09a	0.77±0.06c	0.81±0.06ab
KL2	5.78±0.51c	0.94±0.08a	0.57±0.03dd	0.68±0.04c
KL3	3.69±0.26d	0.67±0.04c	0.61±0.04d	0.76±0.07b
GY1	3.49±0.23d	0.71±0.05bc	0.93±0.08b	0.91±0.09a
GY2	3.22±0.21e	0.87±0.07a	0.56±0.03e	0.69±0.05c
GY3	2.46±0.14f	0.66±0.03bc	0.46±0.02f	0.57±0.04d
QX1	8.11±0.77b	0.61±0.02c	0.90±0.08b	0.72±0.06bc
QX2	11.51±1.22a	0.45±0.03d	1.00±0.11a	0.79±0.05b
QX3	5.22±0.41c	0.65±0.05c	0.87±0.06bc	0.58±0.03d

Note: Data in same column marked with different letter means significant difference ($p < 0.05$).

are affected by the growth environment (including sunlight, climate, and soil factors).

***Nutrients and pH in A. annua rhizosphere soil*:** The growth of *A. annua* at different sites varies with nutrient availability (Aftab *et al.*, 2013; Zhu *et al.*, 2016). The concentrations of total and available N, P, and K, organic matter, and pH at different sampling sites are presented in Table 3. Organic matter was the highest in GY1 sample (139.63 g kg^{-1}) and lowest in QX2 sample (42.01 g kg^{-1}). The average concentration of organic matter in KL and GY samples (112.81 and 119.51 g kg^{-1}, respectively) was significantly higher, when compared with that in QX sample (59.81 g kg^{-1}). Furthermore, in all the sampling sites, the highest total P concentration was 2.25 times higher than the lowest concentration, whereas the highest total K concentration was 2.48 times higher than the lowest concentration. The available N, P, and K nutrients, which are closely related to *A. annua* growth, varied from 12.37 to 46.84, 91.49 to 245.45, and 46.38 to 144.02 mg kg^{-1}, respectively. The pH of the soil samples was alkaline, with values ranging from 7.45 to 8.06 (Table 3), due to high contents of gravels in Karst soils (Table 1). These findings, indicating the diversities of soil nutrients at different sites, revealed the ability of *A. annua* to adapt to complex growth environment.

***Relationship between the concentrations of A. annua phenolics and soil nutrients and pH*:** The concentrations of total and available N, P, and K, concentration of organic matter, and pH affected the soil quality and nutrient absorption by *A. annua* root system, thus affecting the growth of *A. annua*. Furthermore, metabolic accumulation of *A. annua* phenolics was affected by different factors of the growth environment. As shown in Table 4, the concentration of the total polyphenols was significantly negatively correlated with the concentration of organic matter, and significantly positively correlated with available K, indicating that available K can improve metabolic accumulation of *A. annua* phenolics. In contrast, scopoletin was significantly positively correlated with the concentration of organic matter and available P, but significantly negatively correlated with available K, suggesting that organic matter and available P may improve metabolic accumulation of scopoletin in *A. annua*, but available K may inhibit scopoletin accumulation. Furthermore, significant positive correlation was noted between chrysosplenol-D and available K, whereas significant negative correlation was found between chrysosplenetin and available N, indicating that increasing concentration of available K could improve metabolic accumulation of chrysosplenol-D, but decrease the concentration of chrysosplenetin in *A. annua*. These results suggested that the metabolic accumulation of *A. annua* phenolics is affected by soil nutrients.

***Enzymatic activities in A. annua rhizosphere soil*:** The soil enzyme activities reflect the intensity and direction of soil biochemical reaction and material cycling process. As shown in Figure 1, urease, invertase, and phosphatase activities in the soil samples varied from 74.16 to 311.27 μg NH_4^+-N/g/24 h, from 24.12 to 10.20 mg Glucose/g/24 h, and from 0.38 to 1.93 mg phenol/g/24 h, respectively.

Table 3. The concentrations of total and available N, P, and K, organic matter, and pH at different sampling site (mean±SE, n = 3).

Site codes	Organic matter (g kg^{-1})	Total N (g kg^{-1})	Available N (mg kg^{-1})	Total P (g kg^{-1})	Available P (mg kg^{-1})	Total K (g kg^{-1})	Available K (mg kg^{-1})	pH
KL1	114.16± 0.42b	1.44±0.12d	80.54±7.06d	1.29±0.10d	15.32±1.04e	10.05±0.57cd	93.03±8.15c	8.04±0.47a
KL2	100.71± 0.73b	1.21±0.08e	101.93±1.99c	1.13±0.07d	12.37±0.71f	7.22±0.47f	46.38±2.73e	7.54±0.36a
KL3	123.57±10.7ab	2.73±0.31a	91.49±8.93c	2.54±0.11a	14.16±1.23e	7.92±0.41e	68.00±5.36d	7.56±0.29a
GY1	139.63±13.8a	2.43±0.25ab	144.24±0.97b	2.30±0.22a	61.64±5.69a	8.34±0.74e	118.38±10.9b	7.45±0.36a
GY2	111.72±9.06b	1.97±0.14d	109.88±8.53c	1.90±0.13b	50.94±4.01b	11.77±1.36c	63.20±4.35d	8.06±0.39a
GY3	107.18±7.66b	2.15±0.20bc	96.73±5.97c	1.69±0.15c	46.84±3.26b	13.73±1.45bc	103.62±10.1bc	7.57±0.27a
QX1	64.81±4.89c	0.93±0.07f	207.36±4.63a	1.79±0.11bc	40.69±2.31c	17.94±1.72a	114.74±10.4b	7.79±0.44a
QX2	42.01±2.29d	0.83±0.04f	152.94±1.88b	1.71±0.08c	28.50±1.67d	12.52±0.71c	144.02±17.3a	7.64±0.52a
QX3	72.62±6.30c	0.87±0.04f	245.45±3.97a	1.90±0.16b	40.16±2.86c	14.39±1.03b	94.51±6.39c	7.92±0.37a

Note: Data in same column marked with different letter means significant difference ($p < 0.05$).

Table 4. The correlation between concentrations of *A. annua* phenolics and soil nutrients and pH (n = 27).

Phenolics	Organic matter	Total N	Available N	Total P	Available P	Total K	Available K	pH
Total polyphenols	-0.707**	-0.241	0.362	-0.002	0.033	0.315	0.534**	-0.012
Scopoletin	0.562**	0.273	-0.247	-0.123	0.435*	-0.371	-0.427*	-0.225
Chrysosplenpl-D	-0.127	-0.165	0.230	-0.152	0.012	0.042	0.471*	-0.073
Chrysosplenetin	0.212	0.129	-0.400*	-0.136	-0.055	-0.293	0.139	-0.101

Note: * and ** indicate significant and highly significant correlation at different sites at $p < 0.05$ and $p < 0.01$.

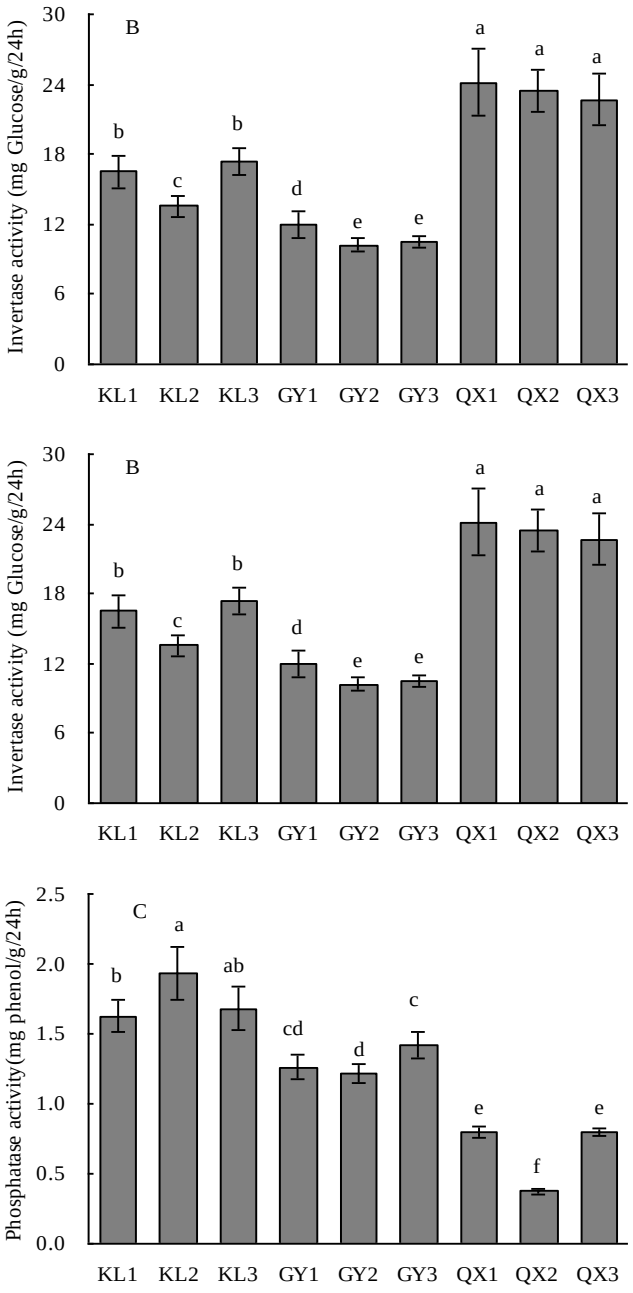

Figure 1. Activity of enzymes in soil from *A. annua* (A: Urease, B: Invertase, C: Phosphatase). Note: Different letters indicate significant difference at different sites at $p < 0.05$. The error bar indicates the standard error.

The urease activity was highest in KL3 sample and lowest in QX1 sample, with the former exhibiting 4.20 times higher activity than the latter. The average urease activity in the three sampling regions was in the following order: KL (241.96 μg NH_4^+-N/g/24 h) > GY (172.57 μg NH_4^+-N/g/24 h) > QX (86.61 μg NH_4^+-N/g/24 h) (Figure 1A). The average invertase activity was highest in QX sample (23.42 mg Glucose/g/24 h), when compared with that in KL and GY samples (15.81 and 10.91 mg Glucose/g/24 h, respectively). However, no difference in the invertase activity was observed among the three QX samples (QX1, QX2 and QX3), whereas GY1 sample showed significantly higher invertase activity than GY2 and GY3 samples, and KL2 sample presented significantly lower invertase activity than KL1 and KL3 samples (Figure 1B). The average phosphatase activity was similar to that of ureases among the different sampling sites as follows: KL (1.75 mg phenol/g/24 h) > GY (1.30 mg phenol/g/24 h) > QX (0.66 mg phenol/g/24 h) (Figure 1C). The diversities of urease, invertase, and phosphatase activities in the soil samples and the different concentrations of *A. annua* phenolics (Table 2) in the samples obtained from different sites illustrated that soil types and geographic conditions influence soil enzyme activities, *A. annua* growth, and metabolic accumulation of *A. annua* phenolics.

***Relationship between concentration of A. annua phenolics and soil enzyme activities*:** To understand the relationship between *A. annua* phenolics and soil enzymes, analysis of the correlation between these two factors is necessary. As shown in Table 5, the concentration of total polyphenols was significantly positively correlated with invertase activity, implying that invertase can supply C and energy sources for soil microorganisms and promote their growth, which in turn activate soil nutrients that are favorable for *A. annua* growth and metabolic accumulation of *A. annua* phenolics. However, the concentration of total polyphenols was significantly negatively correlated with urease and phosphatase activities, indicating that available N and P in the soil may not be primarily activated by urease and phosphatase activities. Furthermore, scopoletin concentration was significantly positively correlated with phosphatase activity, while chrysosplenol-D was significantly positively correlated with invertase activity and significantly negatively correlated with phosphatase activity. These results suggested that the metabolic accumulation of *A. annua* phenolics is closely related to the enzymatic activities in the *A. annua* rhizosphere soil.

Table 5. The correlation between concentrations of *A. annua* phenolics and soil enzymes ($n = 27$).

Phenolics	Urease	Invertase	Phosphatase
Total polyphenols	-0.475*	0.696**	-0.612**
Scopoletin	0.270	-0.336	0.651**
Chrysosplenpl-D	-0.068	0.489*	-0.431*
Chrysosplenetin	0.273	-0.193	0.124

Note: * and ** indicate significant and highly significant correlation at different sites at $p < 0.05$ and $p < 0.01$.

***Microbial biomass in A. annua rhizosphere soil*:** The microbial biomass C and N in soil are regarded as the key indices in the evaluation of soil available nutrients and microorganism activities, because they are the significant sources of available C and N (Wu and Ai, 2008).

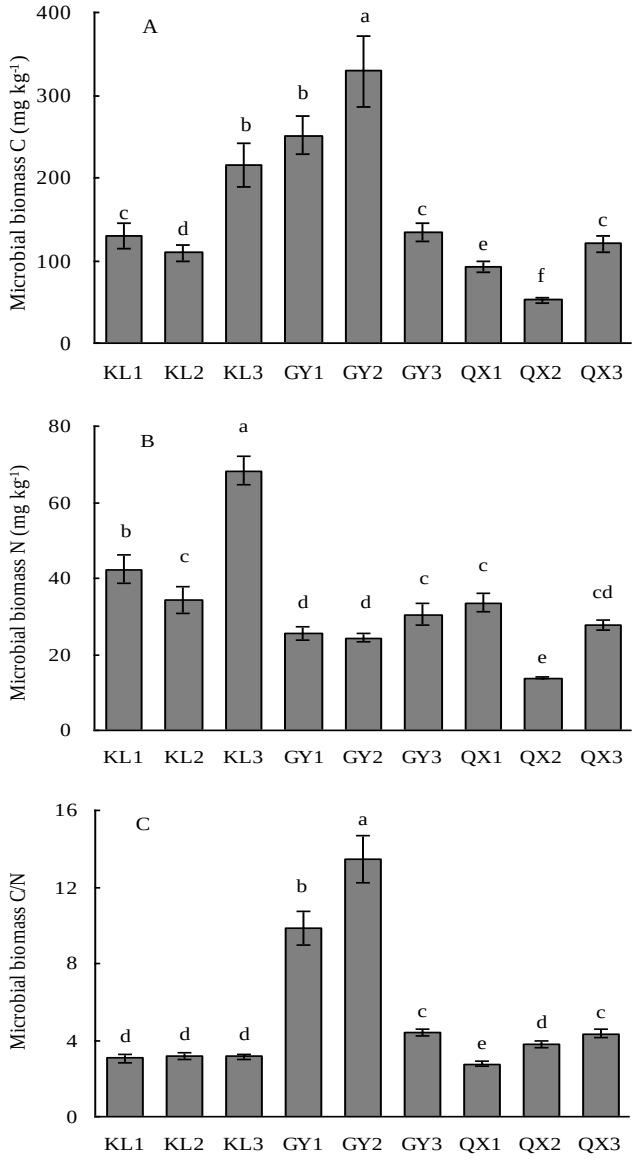

Figure 2. Microbial biomass C, N and C/N in soil from *A. annua* (A: Microbial biomass C, B: Microbial biomass N, C: Microbial biomass C/N). Note: Different small letters indicate significant difference among different sites at $p < 0.05$. The error bar indicates the standard error.

The microbial biomass C in the different samples varied from 51.94 to 328.58 mg kg^{-1} owing to environmental and microbial diversities. The microbial biomass C in GY2 sample was significantly higher than that in other samples, and was 6.33 times higher than that in QX2 sample, which presented the lowest microbial biomass C. In addition, the average microbial biomass C in GY sample (238.13 mg kg^{-1}) was significantly higher than that in KL and QX samples (151.07 and 88.06 mg kg^{-1}, respectively), implying diverse biological availability of rhizosphere nutrients and energy, which are enhanced by the release of organic matter from root exudate or stubble decay. In contrast to microbial biomass C, microbial biomass N was the highest in KL3 sample (68.35 mg kg^{-1}) and lowest in QX2 sample (13.78 mg kg^{-1}). The average microbial biomass N in KL sample (48.30 mg kg^{-1}) was significantly higher than that in GY and QX samples (26.76 and 25.07 mg kg^{-1}, respectively), and was significantly different among KL1, KL2, and KL3 samples, implying the diverse availability of soil nutrients and microbial activities in *A. annua* rhizosphere soil. The average microbial biomass C/N in the samples was similar to that of microbial biomass C, and varied from 2.74 to 13.47. The highest microbial biomass C/N was found in GY2 sample, which was 4.92 times higher than that in QX1 sample, which showed the lowest microbial biomass C/N, suggesting the variation in available N released by microbial biomass transformation in *A. annua* rhizosphere soil.

***Relationship between concentration of A. annua phenolics and soil microbial biomass C, N, and C/N*:** Besides soil enzymes, the metabolic accumulation of *A. annua* phenolics is also closely related to soil microbial biomasses. As shown in Table 6, the concentration of total polyphenols was significantly positively correlated with the microbial biomass C. The concentration of scopoletin was significantly positively correlated with microbial biomass C and C/N, whereas the concentration of chrysosplenetin was significantly positively correlated with microbial biomass C. These findings implied that soil microbial biomass influenced the nutrient absorption by *A. annua* roots from soils, subsequently affecting the metabolic accumulation of *A. annua* phenolics (including polyphenols, scopoletin, chrysosplenol-D, and chrysosplenetin).

Table 6. The correlation between concentrations of *A. annua* phenolics and microbial biomass C, N and C/N of soils (n = 27).

Phenolics	Microbial biomass C	Microbial biomass N	Microbial biomass C/N
Total polyphenols	0.707**	-0.321	-0.280
Scopoletin	0.562**	0.227	0.418*
Chrysosplenpl-D	-0.327	-0.31	0.005
Chrysosplenetin	0.412*	0.102	0.123

Note: * and ** indicate significant and highly significant difference at different sites at $p < 0.05$ and $p < 0.01$.

***Conclusions*:** The present study investigated the relationship between the concentrations of *A. annua* phenolics and nutrients, enzymes, and microbial biomass in the *A. annua rhizosphere soil*. The nutrients, enzymes, and microbial biomass in the soil were found to be the major factors affecting the metabolic accumulation of *A. annua* phenolics. These findings suggest that the availability of soil nutrients and transformation of soil N and P are promoted by *A. annua* through activation of rhizosphere enzymes and microorganisms. This is advantageous for the adaptation of *A. annua* to a wide range of soil environments and influences the metabolic accumulation of *A. annua* phenolics. Moreover, the results of the present study provide theoretical basis for improving the quality of *A. annua*.

***Acknowledgments*:** This research was supported by the National Natural Science Foundation of China (No. 41461053) and the Projects of Scientific Research of Doctors supported by Guizhou Normal University.

REFERENCES

Aftab, T., M. Naeem, M. Idrees, M.M.A. Khan, Moinuddin and L. Varshney. 2013. Cumulative role of irradiated sodium alginate and nitrogen fertilizer on growth, biochemical processes and artemisinin production in *Artemisia annua*. Ind. Crops Prod. 50:874-881.

Alina, K.P. 2011. Trace Elements in Soil and Plants, 4th Ed. CRC Press, Boca Raton, USA.

Awan, A.A., M. Zubair, S. Ali, I. Jan, A. Iqbal and A. Ahmad. 2015. The effect of different climatic zones on total phenolics and fatty acid profile of various olive cultivars. Pak. J. Agri. Sci. 52:359-364.

Bao, S.D. 2005. Soil Agro-chemistrical Analysis. China Agriculture Press, Beijing, China.

Brisibe, E.A., E.U. Umoren, F. Brisibe, P.M. Magalhäes, J.F.S. Ferreira, D. Luthria, X.L. Wu and R.L. Prior. 2009. Nutritional characterisation and antioxidant capacity of different tissues of *Artemisia annua* L. Food Chem. 115:1240-1246.

Chukwurah, P.N., E.A. Brisibe and A.N. Osuagwu. 2014. Protective capacity of *Artemisia annua* as a potent antioxidant remedy against free radical damage. Asian Pac. J. Trop. Biomed. 4:92-98.

Committee of Chinese Pharmacopoeia. 2015. Chinese Pharmacopoeia. 1st ED. China Medical Science and Technology Press, Beijing, China.

Guan, S.Y. 1986. Soil enzymes and its research methods. Chinese Agricultural Press, Beijing, China.

Hu, N., H. Li, Z. Tang, Z. Li, G. Li, Y. Jiang, X. Hu and Y. Lou. 2016. Community size, activity and C:N stoichiometry of soil microorganisms following reforestation in a Karst region. Eur. J. Soil Biol. 73:77-83.

Jessing, K.K., N. Cedergreen, P. Mayer, L. Libous-Bailey, B.W. Strobel, A. Rimando and S.O. Duke. 2013. Loss of artemisinin produced by *Artemisia annua* L. to the soil environment. Ind. Crops Prod. 43:132-140.

Jorge, F.S.F., L.L. Devanand, S. Tomikazu and H. Arne. 2010. Flavonoids from *Artemisia annua* L. as antioxidants and their potential synergism with artemisinin against malaria and cancer. Molecules 15:3135-3170.

Kuklová, M., H. Hniličková, J. Kukla and F. Hnilička. 2015. Environmental impact of the Al smelter on physiology and macronutrient contents in plants and Cambisols. Plant Soil Environ. 61:72-78.

Luo, S.Q., J.G. Huang and L. Yuan. 2014. Nutrients and microorganisms in soils with wild *Artemisia annua* L. Acta Pedol. Sin. 51:868-879.

Luo, S.Q., L. Yuan, Y.K. Wu and J.G. Huang. 2013. Effect of fertilization on phenolics and antioxidant activities of *Artemisia annua*. Chin. J. Chin. Mater. Med. 10:1493-1499.

Martínez-Blanco, J., A. Antón, J. Rieradevall, M. Castellari and P. Muñoz. 2011. Comparing nutritional value and yield as functional units in the environmental assessment of horticultural production with organic or mineral fertilization. Int. J. Life Cycle Assess. 16:12-26.

Mehdi, S.M., M. Sarfraz, M.A. Qureshi, M. Ilyas, M.A. Zaka, M.A. Qazi and H.U. Rafa. 2018. Site-specific phosphorus management with inorganic fertilize and municipal solid waste compost application in salt affected soil. Pak. J. Agri. Sci. 55:103-110.

Peng, W.X., K.L. Wang, T.Q. Song, F.P. Zeng and J.R. Wang. 2008. Controlling and restoration models of complex degradation of vulnerable Karst ecosystem. Acta Ecol. Sin. 28:811-820.

Tischera, A., E. Blagodatskaya and U. Hamer. 2015. Microbial community structure and resource availability drive the catalytic efficiency of soil enzymes under land-use change conditions. Soil Biol. Biochem. 89:226-237.

Wang, L.C., D.W. Lee, P. Zuo, Y.K. Zhou and Y.P. Xu. 2014. Karst environment and eco-poverty in southwestern China: A case study of Guizhou Province. Chin. Geogr. Sci. 14:21-27.

Wu, J.G. and L. Ai. 2008. Soil microbial activity and biomass C and N content in three typical ecosystems in Qilian Mountains. Chin. J. Plant Ecol. 32:465-476.

Yao, H.Y. and C.Y. Huang. 2006. Ecology and study method of soil microorganism. Chinese Science Press, Beijing, China.

Zhang, J.J., H. Li, H.J. Gao, P. Zhu, Q. Gao and L.C. Wang. 2014. Effects of long-term fertilization and cropping regimes on total nitrogen and organic nitrogen forms in a

Mollisol of Northeast China. Plant Soil Environ. 60:544-549.

Zhu, C.W., Q.L. Zeng, H.Y. Yu, S.J. Liu, G.Q. Dong and J.G. Zhu. 2016. Effect of elevated CO_2 on the growth and macronutrient (N, P and K) uptake of annual wormwood (*Artemisia annua* L.). Pedosphere 26:235-242.