

# Changes characteristics of soil microbial biomass carbon,nitrogen and enzyme activity of Panax notoginseng under optimal management of water and fertilizer

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## Abstract

There is a lack of understanding of the dynamic characteristics of carbon, nitrogen, and enzyme activity of soil microbial load of Panax notoginseng in water-fertilizer intercrops. In this study , we reveal that different water and fertilizer regulations affect microbial biomass carbon, nitrogen, and enzyme activities. As the study object, we set up 3 irrigations,4 fertilization levels, and 1 control in micro-sprinkler irrigated Panax notoginseng farmland, Luxi County, Yunnan Province from 2018 to 2020. The findings demonstrated that under the same water and fertilizer management, the carbon, nitrogen, and enzyme activities of Panax notoginseng's soil increased and then decreased with increasing fertility time, in descending order of flowering, fruiting, seedling, and rooting periods.. The maximum value is reached during the flowering period ,while the minimum value is reached during the rooting period. The soil microbial carbon and nitrogen contents ranged from 0.49 to 1.05 g.kg<sup>-1</sup> and from 14.98 to 66.21 mg.kg<sup>-1</sup>, respectively, and soil sucrose enzyme activity was the largest, ranging from 17.12 to 68.79 mg.kg<sup>-1</sup>.d. <sup>-1</sup>. The soil microbial carbon , nitrogen and enzyme activities of Panax notoginseng increased with the rate of water and fertilizer application under different water and fertilizer management. The soil microbial carbon , nitrogen and enzyme activities of Panax notoginseng at the flowering period were the largest. The soil microbial carbon and nitrogen activities of Panax notoginseng increased with the increase of irrigation and fertilizer application, whereas the soil microbial carbon and nitrogen activities of W3F4 increased by 0.41 g.kg<sup>-1</sup> and 39.52 mg.kg<sup>-1</sup> respectively compared with W1F1. Soil urease, sucrase, acid phosphatase, and catalase activities were the highest in W3F4, with increases of 44.26%, 61.51%, 42.56, and 32.25% respectively compared to W1F1. There was a significant positive correlation between soil microbiomass carbon and nitrogen and enzyme activity under different water and fertilizer management. Soil microbiomass carbon and nitrogen content determined soil enzyme activity. The entropy value method combined with the TOPSIS method was used to analyze the optimal program fit Ci of soil microbial biomass carbon, nitrogen, and enzyme activity under different water and fertilizer optimization management and at different fertility periods. The results showed that the Ci values were F4, F3, F2, and F1 in descending order under the same irrigation level treatment.The Ci values decreased and then increased with increasing irrigation water under the same fertilization level treatment. The carbon, nitrogen, and enzyme activities of the soil's microbial biomass were successfully controlled when Panax notoginseng was treated with W2F4 during the rooting period and W3F4 during the seedling, flowering, and fruiting periods. This study is an essential guideline for water and fertilizer regulation of Panax notoginseng and its yield quality improvement.

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**Abstract:** There is a lack of understanding of the dynamic characteristics of carbon, nitrogen, and enzyme activity of soil microbial load of *Panax notoginseng* in water-fertilizer intercrops. In this study, we reveal that different water and fertilizer regulations affect microbial biomass carbon, nitrogen, and enzyme activities. As the study object, we set up 3 irrigations, 4 fertilization levels, and 1 control in micro-sprinkler irrigated *Panax notoginseng* farmland, Luxi County, Yunnan Province from 2018 to 2020. The findings demonstrated that under the same water and fertilizer management, the carbon, nitrogen, and enzyme activities of *Panax notoginseng*'s soil increased and then decreased with increasing fertility time, in descending order of flowering, fruiting, seedling, and rooting periods. The maximum value is reached during the flowering period, while the minimum value is reached during the rooting period. The soil microbial carbon and nitrogen contents ranged from 0.49 to 1.05 g.kg<sup>-1</sup> and from 14.98 to 66.21 mg.kg<sup>-1</sup>, respectively, and soil sucrose enzyme activity was the largest, ranging from 17.12 to 68.79 mg.kg<sup>-1</sup>.d<sup>-1</sup>. The soil microbial carbon, nitrogen and enzyme activities of *Panax notoginseng* increased with the rate of water and fertilizer application under different water and fertilizer management. The soil microbial carbon, nitrogen and enzyme activities of *Panax notoginseng* at the flowering period were the largest. The soil microbial carbon and nitrogen activities of *Panax notoginseng* increased with the increase of irrigation and fertilizer application, whereas the soil microbial carbon and nitrogen activities of W3F4 increased by 0.41 g.kg<sup>-1</sup> and 39.52 mg.kg<sup>-1</sup> respectively compared with W1F1. Soil urease, sucrase, acid phosphatase, and catalase activities were the highest in W3F4, with increases of 44.26%, 61.51%, 42.56, and 32.25% respectively compared to W1F1. There was a significant positive correlation between soil microbiomass carbon and nitrogen and enzyme activity under different water and fertilizer management. Soil microbiomass carbon and nitrogen content determined soil enzyme activity. The entropy value method combined with the TOPSIS method was used to analyze the optimal program fit Ci of soil microbial biomass carbon, nitrogen, and enzyme activity under different water and fertilizer optimization management and at different fertility periods. The results showed that the Ci values were F4, F3, F2, and F1 in descending order under the same irrigation level treatment. The Ci values decreased and then increased with increasing irrigation water under the same fertilization level treatment. The carbon, nitrogen, and enzyme activities of the soil's microbial biomass were successfully controlled when *Panax notoginseng* was treated with W2F4 during the rooting period and W3F4 during the seedling, flowering, and fruiting periods. This study is an essential guideline for water and fertilizer regulation of *Panax notoginseng* and its yield quality improvement.

**Keywords:** *Panax notoginseng*; Optimal management of water and fertilizer; Microbial biomass of Carbon and Nitrogen; Soil enzyme activity.

Soil microbial biomass (SMB), an active component of soil organic matter, plays a key role in the regulation of soil ecosystem and its function, serving as one of the most important biological indicators for the evaluation of the soil comprehensive quality (Raiesi et al., 2015; Evangelou et al., 2021). Soil MBC and MBN determine soil fertility and are important source of soil organic carbon and available nitrogen (Yu et al., 1999; Zhou et al., 2002). Soil enzyme activity is derived from soil microorganisms and plant root exudates and is sensitive to different fertilization responses (Yang et al., 2020). It is very active in soil ecosystems (Luo et al., 2014; Hill et al., 2012) and plays a vital role in nutrient cycling (nitrogen fixation, phosphorus adsorption and desorption, and potassium release, etc.) as well as in soil structure maintenance and crop yield (Burns et al., 2013; Zhao et al., 2016). It is a comprehensive soil fertility biological index (Zhang et al., 2015). As a catalyst for soil organic matter decomposition and nutrient conversion cycling, soil enzyme has a close relationship with soil nutrient content and microbial biomass carbon and nitrogen (Wang et al., 2016). Soil comprehensive fertility

is determined by soil microbial biomass carbon and nitrogen and enzyme activity.

Fertilization and irrigation had significant effects on soil microbial biomass C and N and enzyme activity. The research by Jiang et al. showed that irrigation improved soil enzyme activity(Wang et al.,2017;Calderon et al.,2016), and the research on soil microbial characteristics, soil enzyme activity, and nutrient regulation in water-saving irrigation winter wheat fields by Ye Deling et al. showed that soil microbial characteristics and soil enzyme activity were regulated by irrigation and were closely related to the cyclic transformation of soil C and N nutrients(Ye et al.,2016). Li et al. found that nitrogen application significantly increased soil MBC and MBC: MBN(Li et al.,2018). Xu et al. studied the effects of fertilization on soil organic carbon (SOC) content, soil microbial biomass carbon (SMC), and soil microbial biomass nitrogen (SMN) and found that the yields of early and late rice treated with 30% organic matter +70% inorganic fertilizer, 60% organic matter +40% inorganic fertilizer and straw+inorganic fertilizer were higher than those of the early and late rice treated with inorganic fertilizer or no fertilizer(Xu et al.,2017). HAO et al. studied the effects of long-term application of inorganic fertilizer and organic fertilizer on the organic matter and microbial biomass of three subtropical rice soil, and the results showed that the mixed application of inorganic fertilizer with manure or straw improved the organic matter and microbial biomass of the subtropical paddy soil(Hao et al.,2008). Bach E.M. et al. showed that the microbial activity in the fertilized grassland was higher than that in the non-fertilized soil, and the addition of nitrogen in the fertilized grassland enhanced the soil microbial biomass and enzyme activity(Bach et al.,2015).

There have been many studies on the effects of various farmland management methods on soil microbial biomass C-N and enzyme activities, but none have been quantitative. Particularly, *Notoginseng*, a medicinal plant, has not received much study. The interaction of water and fertilizer has little effect on the rhizosphere secretion of *Panax notoginseng* and soil microbial biomass C-N and enzyme activities, thereby affecting the farmland soil microbiological properties of *Panax notoginseng*. At the moment, research on the interaction of water and fertilizer is primarily focused on regulating the improvement of soil saline-alkali land in dryland farmland in the north, as well as crop yield and quality(Li et al.,2016;Zheng et al.,2020). *Panax notoginseng* research is primarily focused on single factors such as cultivation mode,continuous cropping obstacle of *Panax notoginseng*, accumulation and adsorption of heavy metals in soil,and fertilizer element ratio(Tang et al.,2020). Few studies have been conducted to investigate the effects of optimal water and fertilizer management on soil microbial biomass C and N and enzyme activities of *Panax notoginseng*, as well as the regulation of the combination of irrigation and fertilization on plant growth, yield, and quality of *Panax notoginseng*. Soil microbial biomass C-N and enzyme activities characterized soil fertility. It was determined that optimal water and fertilizer management was conducive to the growth, yield, and quality improvement of *Notoginseng* by investigating the soil microbial biomass C-N and enzyme activities under optimal water and fertilizer management. In this study, a randomized zonal experimental design with different water and fertilizer interactions was used to optimize the management of *Panax notoginseng* through three years of growth from 2018 to 2020. In this experiment, the effect of different water and fertilizer management on soil microbial carbon, nitrogen and enzyme activities of *Panax notoginseng* was analyzed, and the mechanism of this management on soil microbial carbon, nitrogen and enzyme activity response was investigated. The results can provide technical support for the efficient use and management of water and fertilizer in *Panax pseudoginseng* farmland, as well as important practical significance for guiding the high yield and quality cultivation of *Panax notoginseng*.

## 1 Materials and methods

### Test design and sample collection

The experiment was conducted in the typical planting area of *Panax notoginseng* in Dali Shu Village, luxi county City, Honghe Prefecture, Yunnan Province from 2018 to 2020. The growth period of *Panax notoginseng* was divided into the rooting period (November January of the following year), the seedling period (February April), the flowering period (May July), and the fruiting period (August October). *Panax notoginseng* was covered with a double-layer sun-shading net. To avoid the influence of natural rainfall

on irrigation amount, plastic film was used to cover and discharge the rainfall, and the excess rainfall was timely discharged from the test area. Micro-sprinkler irrigation fertilization was adopted. Irrigation and fertilization were conducted simultaneously once a month. The fertilizer was soluble organic fertilizer. The micro-sprinkler flow was controlled according to the experimental design to meet the design criteria for fertilization and irrigation in different plots. According to the high-yield and high-efficiency fertigation system for planting *Panax notoginseng* in the local area, two factors (F) and (W) were applied in the experiment. The former includes four levels, namely, F1(3.20kg/667m<sup>2</sup>), F2(4.80kg/667m<sup>2</sup>), F3(6.20kg/667m<sup>2</sup>), and F4 (8.00 kg/667 m<sup>2</sup>); The latter three levels were W1(40% field water holding capacity), W2(60% field water holding capacity) and W3(80% field water holding capacity), one control. A total of 13 treatments were set, which were W1F1, W1F2, W1F3, W1F4, W2F1, W2F2, W2F3, W2F4, W3F1, W3F2, W3F3, W3F4 and CK, respectively. CK was the no-fertilization and no-irrigation control. Each processing three repeat, each community 16.70m long, 1.50m wide, ditching ridging before planting, the groove depth of 30cm, the groove width of 40cm, to ensure that the water is not leaking, not diffuse groove, such as regular weed field management measures.

The sampling was conducted on the third day after irrigation and fertilization in December of each year, March, June, and September of the following year from 2018 to 2020. During the experiment, a 1m×1m sampling point was set in each residential area to remove soil surface litter and gravel around the sampling point at the sampling depths of 0~20cm and 20~40cm, respectively. The soil samples were put into a self-sealing bag for labeling, sealed and stored, and then taken back to the laboratory. The fresh soil sample was divided into two parts. One part was used to measure soil water content and microbial carbon and nitrogen content. The other part was spread on kraft paper and air-dried naturally to remove roots, leaves, and gravel. Soil enzyme activity was tested after grinding and sieving. The German-American water-soluble organic fertilizers with N[?]21%, P<sub>2</sub>O<sub>5</sub>[?]21%、K<sub>2</sub>O[?]21%, humic acid [?]6%, B[?]0.1%, and Mo[?]0.007% were selected. The basic physical and chemical properties of the soil before the test are shown in Tab.1.

**Table 1 Basic physical and chemical properties of soil**

Organic carbon g[?]kg <sup>-1</sup>	Total nitrogen g[?]kg <sup>-1</sup>	Total potassium g[?]kg <sup>-1</sup>	Total Potassium g[?]kg <sup>-1</sup>	Available potassium mg[?]kg <sup>-1</sup>	Nitrate nitrogen mg[?]kg <sup>-1</sup>	Ammonium nitrogen mg[?]kg <sup>-1</sup>	Available potassium mg[?]kg <sup>-1</sup>	pH	Field water holding capacity %
14.33	0.98	0.37	14.79	316.8	8.35	19.15	11.65	6.34	42.3

## 1.2 Determination method

The chloroform fumigation extraction method was used for the determination of soil microbial biomass carbon and nitrogen. The chloroform-fumigated and non-fumigated soils were extracted with 0.5mol/L K<sub>2</sub>SO<sub>4</sub> solution at the soil/liquid ratio of 1: 4. The organic carbon content in the extraction solution was determined by FeSO<sub>4</sub> solution titration with a conversion coefficient of 0.38, and the organic nitrogen content in the extraction solution was determined by ninhydrin colorimetric method with a conversion coefficient of 5.00(Li et al.,2008).The measurement of fumigation and non-fumigation was repeated 3 times, and the mean value was taken.

Soil enzyme activity was determined by referring to Soil Enzyme and Its Research Method(Guan et al.,1986).Catalase (Cat) was measured by KMnO<sub>4</sub> titration as a ml number of 1g.h<sup>-1</sup> soil consuming 0.1 mol.L<sup>-1</sup>KMnO<sub>4</sub>.Urease (Ure) using phenol-sodium hypochlorite colorimetric method, in 1g of soil after 1d culture generated NH<sub>3</sub>-N mg number said urease activity; Sucrase (Sue) was colorimetric with 3,5-dinitrosalicylic acid and expressed as sucrase activity in milligrams of glucose formed in 1g of soil after 1d of culture. Acid Phosphatase (Acp) activity was determined using a disodium phosphate colorimetric method using milligrams of phenol released from 1g of soil 1d later. Each soil sample was measured three times, and

the average value was taken. The soil moisture water content was determined by the drying method.

### Soil microbial biomass carbon and nitrogen and enzyme activity evaluation calculation

In this paper, the entropy method is used to determine the weight of each index. Due to the differences between each index, in order to eliminate the dimension of index data and the positive and negative effects of the index, the original data is standardized to form a new data column. The following formula is the entropy method to calculate the index weight (Huang et al.,2021;Lu et al.,2019):

(1) Data standardization

(1)

Where:  $x_{ij}$  is the value of indicator of its sample ( $i=1,2,\dots,n; j=1,2,\dots,n$ );  $x_{ij}^{\max}$  and  $x_{ij}^{\min}$  are the maximum and minimum values of indicator  $j$ , respectively, and  $x_{ij}^{\max}$  is the normalized  $x_{ij}$ , which were the standardized indicators of soil microbial carbon and nitrogen content and enzyme activity under different water and fertilizer optimal management.

(2) Calculate the weight of the sample data under the indicator for that indicator :

(2)

(3) TOPSIS calculator

Construction of original evaluation matrix:

There are  $m$  evaluation objects,  $n$  evaluation indicators, the indicators and the corresponding entropy weights multiplied, the data are represented as the original matrix,  $x_{ij}$  is the original data of the  $j$ th indicator of the  $i$ th treatment,  $x_{ij}$  is the original data of the  $j$ th indicator of the  $i$ th treatment:

(3)

Where:  $i=(1,2,\dots,m)$ ,  $j=(1,2,\dots,n)$ ,  $m=13$ , are 13 water and fertilizer treatment trials.  $n=7$ , are indicators of soil enzyme activity, microbial load carbon and nitrogen, and water content .

Normalization matrix. The maximum and minimum values of each column constitute the optimal and inferior vectors respectively:

(4)

The distance between the  $i$ th treatment and the optimal and inferior solutions are:

(5)

The  $i$ th treatment fits to the optimal solution  $C_i$  are:

(6)

Microsoft Excel 2016 and SPSS26.0 were used for statistical analysis of the data, and one-way ANOVA was used to test the differences of soil microbial biomass carbon and nitrogen content and enzyme activity under different water and fertilizer optimal management ( $\alpha=0.05$ ). The entropy method was used to calculate the weight and rank the soil microbial biomass carbon and nitrogen content and enzyme activity. Mantel analysis was performed using R language, and Origin2020b software was used for mapping.

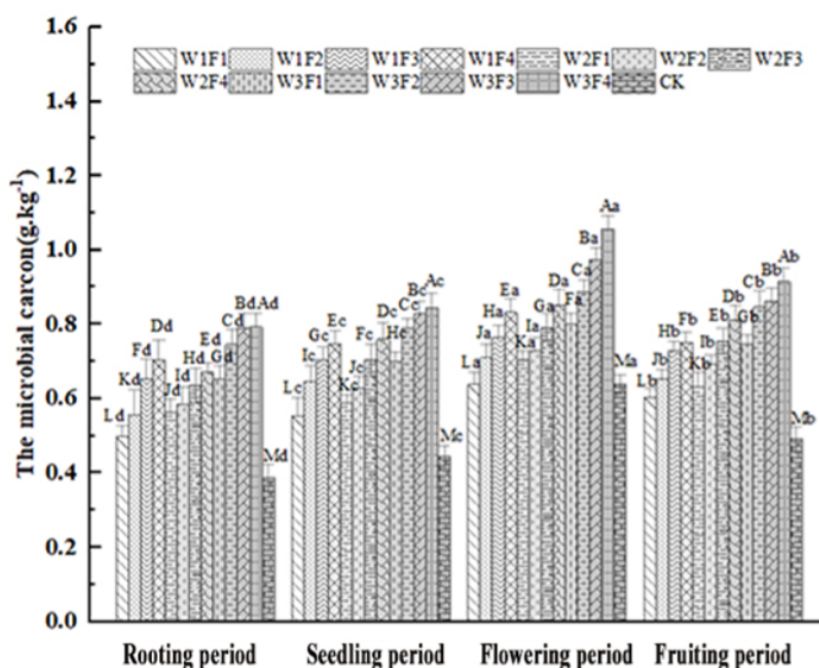
## 2 Result analysis

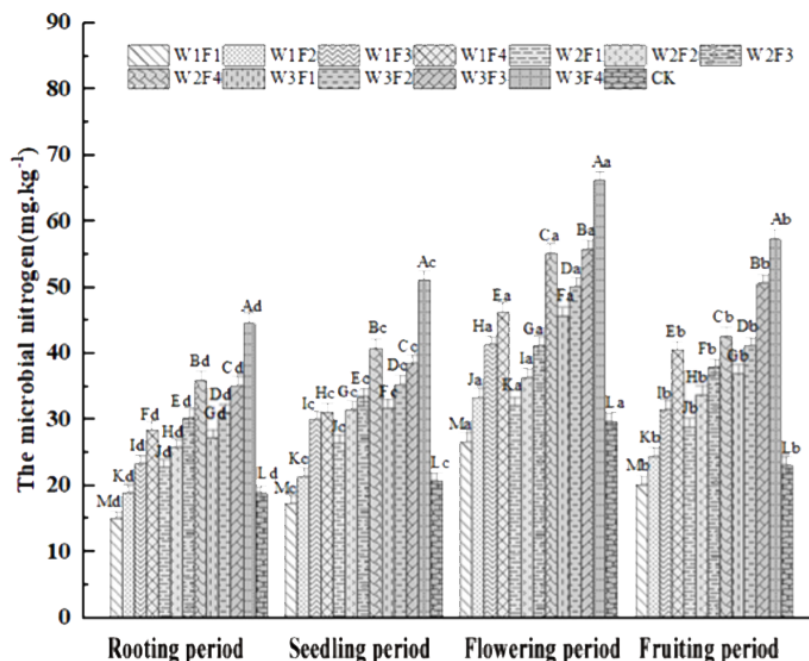
### 2.1 Effects of different water and fertilizer optimal management on soil microbial biomass carbon and nitrogen change characteristics

Under the same optimal management of water and fertilizer, the difference in soil microbial biomass C and N content was significant with different growth periods ( $p<0.05$ ), and the changing trend was increased first and then decreased with time, and the order was flowering, fruiting, seedling and rooting period from big

to small. As shown in Fig. 1, the carbon content of soil microbial biomass ranged from 0.49 to 1.05 g.kg<sup>-1</sup>, increased by 14.37% and 39.59% respectively from rooting period to flowering period, and the difference between the flowering period and rooting period of W3F4 was the largest, 0.26 g.kg<sup>-1</sup>; the difference between the flowering period and rooting period of W1F3 was the smallest, 0.11 g.kg<sup>-1</sup>; the difference between the flowering period and rooting period of CK was 0.25 g.kg<sup>-1</sup>, which was second only to that of W3F4 and ranked the second among all treatments. The soil microbial biomass nitrogen content ranged from 14.98 to 66.21 mg.kg<sup>-1</sup>, increasing by 26.55% and 43.86% respectively from rooting period to flowering period. The difference between the flowering period of W3F4 and the rooting period was the largest, 20.67 mg.kg<sup>-1</sup>, while the difference between the flowering period of W2F1 and the rooting period was 9.23 mg.kg<sup>-1</sup>, the smallest difference for all treatments. The CK flowering period increased by 10.88 mg.kg<sup>-1</sup> compared with the rooting period, ranking 12th among all treatments. Therefore, the soil microbial biomass C and N content of W3F4 changed most in the four growth periods.

Soil microbial biomass C and N content in the same growth period had significant differences with different water and fertilizer optimal management ( $p < 0.05$ ), and the soil microbial biomass C and N content increased with the increase of irrigation amount and fertilizer application amount. It could be seen from Fig. 1 that the soil microbial biomass C and N content of W3F4 in all growth periods was greater than that of other treatments. The soil microbial biomass C of W3F4 at the flowering period increased by 0.41 g.kg<sup>-1</sup>, compared with the minimum value W1F1, and increased by 39.38% compared with CK at the same period. During the seedling period, W3F4 increased by 0.29 g.kg<sup>-1</sup>, compared with the minimum value of W1F1, and W3F4 at the seedling period was 1.9 times that of CK. The soil microbial biomass nitrogen at the flowering period was the maximum, and W3F4 increased by 39.52 mg.kg<sup>-1</sup> compared with the minimum value of W1F1, and increased by 55.08% compared with CK at the same time. However, during the rooting period W3F4 increased by 29.63 mg.kg<sup>-1</sup> compared with the minimum value of W1F1, and W3F4 during the rooting period was 2.36 times as much as CK. In the same growth period, CK was greater than that of W1F1, and the difference in the rooting period was the largest, 3.86 mg.kg<sup>-1</sup>, and the ratio of CK to that of W1F1 was the largest, 20.51% in the rooting period.





**Fig.1 Characteristics of changes in soil microbial biomass C and N under different optimal water and fertilizer management**

Note: Different capital letters indicate significant difference between different water and fertilizer control treatments in the same growth period ( $p < 0.05$ ); Different lowercase letters indicate significant difference between different growth periods under the same water and fertilizer control treatment ( $p < 0.05$ )

## 2.2 Effect of different water and fertilize optimal management on soil enzyme activity change characteristics

Under the same optimal management of water and fertilizer, the activities of soil urease, sucrase, acid phosphatase, and catalase showed significant differences at different growth periods ( $p < 0.05$ ), which increased first and then decreased with the prolongation of the growth period of *Panax notoginseng*, and the activities ranged from large to small in flowering period, fruiting period, seedling period and rooting period. Tab.2 shows that the activities of urease, sucrase, acid phosphatase and catalase are 2.86 9.52  $\text{mg.kg}^{-1}.\text{d}^{-1}$ , 17.12 68.79  $\text{mg.kg}^{-1}.\text{d}^{-1}$ , 2.14 8.2  $\text{mg.kg}^{-1}.\text{d}^{-1}$  and 5.1 9.42  $\text{mg.kg}^{-1}.\text{h}^{-1}$ , respectively. Under the same optimal management of water and fertilizer, the active of urease, sucrase, acid phosphatase, and catalase increased by 30.81% and 46.08%, 31.94% and 44.43%, 34.08% and 59.01%, 12.44% and 37.86% respectively from flowering period to fruiting period. The soil enzyme activities of W3F4 were higher than those of other treatments. The rooting period of urease and catalase activities of W3F4 were 2.14 and 1.67 times of CK, respectively. The flowering period of sucrase and acid phosphatase activities were 1.83 and 2.53 times CK. The soil water content decreased firstly and then increased and then decreased with the prolongation of the growth period. The maximum value appeared in the flowering period, and the minimum value in the seedling period. The difference between the flowering and seedling period of W3F4 was the largest, 4.3%, and that of W2F3 was the smallest, 1.72%.

Under different water and fertilizer optimal management, soil urease, sucrase, acid phosphatase, and catalase activities in the same growth period showed significant differences ( $p < 0.05$ ). Soil urease, sucrase, acid phosphatase, and catalase activities increased with the increase of irrigation amount and fertilizer application amount. Soil urease, sucrase, acid phosphatase, and catalase activities W3F4 at the flowering period were

the largest, increasing by 44.26%, 61.51%, 42.56 and 32.25% respectively as compared with the minimum value W1F1; Under the same water and fertilizer optimal management, the soil enzyme activities increased with the increase of fertilizer application amount, and the activities of urease, sucrase, acid phosphatase and catalase in W3F4 were the highest, which were 1.95, 1.69, 2.32 and 1.47 times of CK, respectively.

**Tab.2 Characteristics of changes in soil enzyme activity under different optimal water and fertilizer management**

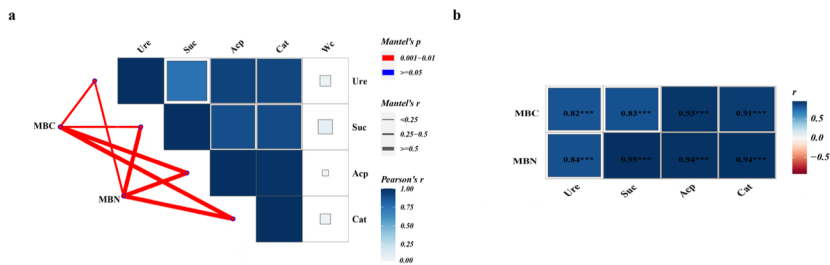
Growth period	Enzyme activity	W1F1	W1F2	W1F3	W1F4	W2F1	W2F2	W2F3	W2F4	W3F1	W3F2	W3F3	W3F4
Rooting period	Urease (mg.g <sup>-1</sup> .d <sup>-1</sup> )	2.86±0.07	3.66±0.08	4.32±0.11	4.79±0.13	5.01±0.14	5.54±0.12	5.76±0.15	5.54±0.13	6.22±0.13	6.90±0.12	7.73±0.16	8.01±0.15
	Sucrase (mg.g <sup>-1</sup> .d <sup>-1</sup> )	17.12±0.82	19.93±0.37	25.54±0.60	30.31±1.07	27.97±0.90	30.89±1.00	34.34±0.82	32.29±1.08	38.78±0.93	44.49±0.63	55.41±0.52	63.23±0.52
	Acid phos-phatase (mg.g <sup>-1</sup> .d <sup>-1</sup> )	2.46±0.42	3.14±0.09	2.60±0.17	3.74±0.10	2.97±0.10	3.17±0.13	3.53±0.14	4.26±0.13	3.13±0.13	3.45±0.06	3.76±0.09	4.48±0.10
	Catalase (mg.g <sup>-1</sup> .h <sup>-1</sup> )	5.36±0.36	6.11±0.13	6.29±0.14	6.95±0.12	7.99±0.12	8.39±0.15	9.93±0.07	7.09±0.12	8.17±0.18	8.73±0.16	8.73±0.14	9.21±0.15
	Moisture content(%)	26.46±0.36	26.94±0.26	26.96±0.68	28.12±0.67	27.60±0.50	26.75±0.67	27.85±0.52	28.16±0.48	28.42±0.52	28.01±0.52	27.82±0.72	27.97±0.72
Seedling period	Urease (mg.g <sup>-1</sup> .d <sup>-1</sup> )	3.55±0.13	3.87±0.12	4.78±0.15	5.63±0.13	5.53±0.14	4.47±0.12	5.31±0.17	6.09±0.16	6.54±0.13	7.44±0.17	7.96±0.15	8.54±0.15
	Sucrase (mg.g <sup>-1</sup> .d <sup>-1</sup> )	19.63±1.01	21.63±1.49	29.24±1.62	32.91±1.60	30.32±1.67	35.06±1.38	38.96±1.38	41.11±1.48	44.15±1.28	48.24±0.99	59.88±1.37	63.54±1.37
	Acid phos-phatase (mg.g <sup>-1</sup> .d <sup>-1</sup> )	2.43±0.09	3.21±0.19	4.03±0.17	4.08±0.23	3.29±0.18	3.62±0.13	3.54±0.16	4.92±0.17	3.58±0.17	4.07±0.15	3.38±0.17	4.02±0.17
	Catalase (mg.g <sup>-1</sup> .h <sup>-1</sup> )	5.53±0.14	5.60±0.12	6.39±0.17	7.17±0.15	7.80±0.05	6.47±0.54	6.94±0.13	7.64±0.09	9.76±0.24	6.48±0.24	7.06±0.23	7.88±0.23
	Moisture content(%)	24.88±0.46	26.02±0.32	25.55±0.45	25.73±0.26	25.19±0.43	25.46±0.56	26.59±0.47	27.29±0.47	27.34±0.54	25.33±0.62	25.67±0.25	27.87±0.25
Flowering period	Urease (mg.g <sup>-1</sup> .d <sup>-1</sup> )	5.31±0.16	6.26±0.20	7.24±0.17	7.69±0.27	7.39±0.18	8.96±0.10	7.13±0.28	8.25±0.24	9.46±0.18	8.43±0.20	8.28±0.17	9.53±0.17
	Sucrase (mg.g <sup>-1</sup> .d <sup>-1</sup> )	26.47±1.32	32.49±1.53	39.00±1.52	52.75±1.34	41.82±1.56	46.20±1.06	56.21±1.22	60.71±1.60	64.76±1.58	68.60±1.67	74.17±1.28	78.80±1.28
	Acid phos-phatase (mg.g <sup>-1</sup> .d <sup>-1</sup> )	4.71±0.12	5.23±0.13	5.57±0.16	6.84±0.09	4.95±0.13	5.00±0.14	6.87±0.13	7.70±0.14	5.72±0.27	6.41±0.19	7.39±0.16	8.21±0.21
	Catalase (mg.g <sup>-1</sup> .h <sup>-1</sup> )	6.38±0.13	6.70±0.12	7.18±0.14	8.00±0.13	7.52±0.10	7.92±0.18	8.09±0.18	8.32±0.17	7.79±0.12	8.21±0.13	8.42±0.19	9.42±0.19
	Moisture content(%)	29.18±0.59	29.58±0.38	28.71±0.49	29.28±0.48	28.96±0.96	28.74±0.49	28.86±0.49	29.01±0.80	30.04±0.38	28.83±0.62	28.65±0.59	29.17±0.59
Fruiting period	Urease (mg.g <sup>-1</sup> .d <sup>-1</sup> )	3.86±0.22	5.53±0.26	6.64±0.13	6.97±0.22	4.00±0.15	5.11±0.25	5.05±0.18	7.25±0.24	8.88±0.16	5.55±0.17	7.89±0.37	7.57±0.37
	Sucrase (mg.g <sup>-1</sup> .d <sup>-1</sup> )	23.27±1.26	36.33±1.42	42.75±1.57	57.82±1.28	38.84±1.25	53.11±1.52	52.00±1.94	48.38±1.58	66.66±1.42	68±1.32	83.18±1.22	82.64±1.22



Growth period	Enzyme activity	W1F1	W1F2	W1F3	W1F4	W2F1	W2F2	W2F3	W2F4	W3F1	W3F2	W3F3	W3F4
	Acid phos-phatase (mg.g <sup>-1</sup> .d <sup>-1</sup> )	3.33±0.24	4.03±0.14	4.77±0.15	5.69±0.23	6.71±0.24	8.08±0.25	9.39±0.15	9.92±0.24	10.06±0.25	10.01±0.25	9.92±0.15	10.35±0.15
	Catalase (mg.g <sup>-1</sup> .h <sup>-1</sup> )	5.95±0.26	6.38±0.15	6.88±0.16	7.28±0.16	6.87±0.16	7.26±0.17	7.43±0.27	7.68±0.14	7.13±0.19	7.45±0.22	7.56±0.30	8.17±0.17
	Moisture content(%)	27.33±0.52	28.31±0.32	28.07±0.52	27.70±0.68	28.30±0.67	27.64±0.42	28.79±0.52	28.58±0.62	29.12±0.52	28.17±0.62	28.03±0.42	27.93±0.52

### 2.3 Effect of soil microbial biomass carbon and nitrogen on enzyme activity unde different water and fertilizer optimal management

The Mantel analysis method was used to obtain correlation and explanation values for the relationship between soil microbial biomass carbon and nitrogen and soil enzyme activity under different water and fertilizer optimal management conditions. It could be seen from Fig.2a that the Mantel significant values of soil acid phosphatase, catalase, and microbial carbon were the smallest (Mantel's  $p < 0.01$ ). Soil sucrose, acid phosphatase, catalase, and microbial nitrogen had the least Mantel's  $p < 0.01$ , while sucrose (Sue) had less than 0.05, and urease had less than 0.05. The maximum interpretation values of soil microbial biomass C and N by acid phosphatase and catalase (Mantel's  $r^2[0.5]$ ) indicated that soil microbial biomass C and N content determined soil acid phosphatase and catalase activities, and the maximum interpretation value of soil microbial biomass N by sucrose (Mantel's  $r^2[0.5]$ ) indicated that soil microbial biomass N content determined soil sucrose (Sue) activity. However, Mantel significant and interpreted values of urease and soil microbial biomass carbon and nitrogen were (Mantel's  $p < 0.05$ , Mantel's  $r = 0.25-0.5$ ), which were important factors affecting microbial biomass carbon and nitrogen. The results in Fig.2b showed that soil enzyme activities and soil microbial biomass C and N had different degrees of correlation. The correlation coefficient of soil acid phosphatase and catalase activity to soil microbial biomass carbon and nitrogen were between 0.90 and 1.00, indicating that there was a very significant positive correlation between soil acid phosphatase and catalase activities and soil microbial biomass carbon and nitrogen content ( $p < 0.01$ ), which was consistent with that results in Fig.2a. The correlation coefficient of urease and sucrose activities to soil microbial biomass c and n were 0.80-0.95, indicating that urease and sucrose activities had a significant positive correlation with soil microbial biomass c and n ( $p < 0.05$ ).



**Fig.2 Mantel analysis of soil enzyme activity on soil micro biomass carbon, nitrogen and their ratios**

Note: MBC is microbial biomass carbon, MBN is microbial biomass nitrogen, Ure is soil urease, Sue is soil sucrose, Acp is soil acid phosphatase, Cat is soil catalase.

### 2.4 Effect evaluation of different water and fertilizer optimal management on soil microbial biomass C and N and enzyme activity of *Panax notoginseng* farmland

To comprehensively and systematically reflect the effects of different water and fertilizer optimal management on soil microbial biomass carbon and nitrogen and enzyme activity of *Panax notoginseng* farmland, and avoid the difficulty in evaluating the quality of water and fertilizer management due to a large number of test indexes. The entropy value method combined with TOPSIS method was used to analyze the indicators of soil microbial biomass carbon, nitrogen and enzyme activity at different fertility periods under different water and fertilizer optimization management of *Panax notoginseng*. This method can determine the fit degree  $C_i$  and its ranking for the optimal solution of soil microbial biomass carbon, nitrogen and enzyme activity at different fertility periods under different water and fertilizer optimization management to find the optimal water and fertilizer management solution at different fertility periods. As shown in Tab.3, The greater the value of the optimal solution fit  $C_i$ , the better the microbial biomass carbon, nitrogen and enzyme activity of *Panax notoginseng* soil for that treatment. Under the same irrigation treatment, the  $C_i$  values were F4, F3, F2 and F1 in descending order, and the advantages and disadvantages of microbial biomass carbon, nitrogen and enzyme activity of *Panax notoginseng* soil were F4, F3, F2 and F1 in descending order. Under the same fertilization treatment, the  $C_i$  values decreased and then increased with the increase of irrigation, and the advantages and disadvantages of microbial biomass carbon, nitrogen and enzyme activity of *Panax notoginseng* soil were W2, W1 and W3 in descending order. The maximum  $C_i$  value of 0.836 for the W2F4 treatment and the next highest  $C_i$  value of 0.794 for the W3F4 treatment at the rooting period, and the worst  $C_i$  values of 0.214 and 0.133 for the W1F2 and W1F1 treatments, respectively, for soil microbial biomass carbon, nitrogen and enzyme activity. The maximum  $C_i$  value of 0.806 for the W3F4 treatment and the next highest  $C_i$  value of 0.801 for the W2F4 treatment at the seedling period. The worst soil microbial biomass carbon, nitrogen and enzyme activity treatments were W1F2 and W1F1 with  $C_i$  values of 0.263 and 0.111, respectively. The maximum  $C_i$  value for the W3F4 treatment was 0.733, followed by 0.568 for the W2F3 treatment, and the worst  $C_i$  values for soil microbial biomass carbon, nitrogen and enzyme activity were 0.216 and 0.189 for the W2F1 and W1F1 treatments, respectively. The maximum  $C_i$  value for the W3F4 treatment was 0.835, followed by 0.766 for the W3F3 treatment, and the worst  $C_i$  values for soil The worst soil microbial carbon, nitrogen and enzyme activity treatments were W2F1 and W1F1, with  $C_i$  values of 0.273 and 0.068, respectively. According to the optimal scheme fit  $C_i$  analysis of soil microbial biomass carbon, nitrogen and enzyme activities at different fertility periods under different water and fertilizer optimal management, W2F4 treatment at rooting period, W3F4 treatment at seedling, flowering and fruiting periods were favorable to regulate soil microbial biomass carbon, nitrogen and enzyme activities of *Panax notoginseng* soil and promote root growth.

**Tab.3 Comprehensive evaluation of water and fertilizer control treatments in different growth periods of *Panax notoginseng***

Treatment	Rooting period	Rooting period	Rooting period	Rooting period	Rooting period	Seedling period	Seedling period	Seedling period	Seedling period	Seedling period	Flowering period	Flowering period	Flowering period	Flowering period	Flowering period
	Weight $W_j$	Positive ideal solution	Negative ideal solution	Relative proximity	Rank	Weight $W_j$	Positive ideal solution	Negative ideal solution	Relative proximity	Rank	Weight $W_j$	Positive ideal solution	Negative ideal solution	Relative proximity	Rank
W1F1	0.074	0.366	0.056	0.133	13	0.075	0.361	0.045	0.111	13	0.076	0.348	0.081	0.189	13
W1F2	0.076	0.323	0.088	0.214	12	0.078	0.296	0.106	0.263	12	0.077	0.282	0.153	0.353	9
W1F3	0.078	0.249	0.166	0.400	8	0.078	0.225	0.168	0.428	8	0.077	0.287	0.131	0.312	10
W1F4	0.078	0.144	0.267	0.651	4	0.078	0.185	0.221	0.545	5	0.076	0.191	0.207	0.522	5
W2F1	0.075	0.296	0.126	0.299	10	0.075	0.279	0.122	0.304	10	0.076	0.316	0.087	0.216	12
W2F2	0.074	0.268	0.139	0.342	9	0.074	0.235	0.158	0.402	9	0.075	0.304	0.111	0.267	11
W2F3	0.075	0.157	0.240	0.605	5	0.075	0.148	0.242	0.621	4	0.075	0.183	0.241	0.568	2
W2F4	0.075	0.066	0.336	0.836	1	0.074	0.081	0.319	0.801	2	0.074	0.188	0.238	0.559	3
W3F1	0.078	0.262	0.185	0.414	7	0.077	0.246	0.195	0.443	7	0.076	0.235	0.244	0.509	6
W3F2	0.079	0.197	0.219	0.526	6	0.078	0.215	0.191	0.472	6	0.077	0.239	0.191	0.443	7

Treatment	Rooting period	Rooting period	Rooting period	Rooting period	Rooting period	Seedling period	Seedling period	Seedling period	Seedling period	Seedling period	Flowering period	Flowering period	Flowering period	Flowering period	Flowering period
W3F3	0.079	0.092	0.316	0.774	3	0.078	0.132	0.282	0.681	3	0.077	0.217	0.259	0.544	4
W3F4	0.077	0.097	0.374	0.794	2	0.076	0.087	0.362	0.806	1	0.077	0.126	0.346	0.733	1
CK	0.068	0.358	0.109	0.233	11	0.069	0.337	0.126	0.272	11	0.074	0.311	0.199	0.392	8

### 3 Discussion

#### 3.1 Effect mechanism of optimal water and fertilizer management on soil microbial biomass C and N of *Panax notoginseng*

This study found significant differences ( $p < 0.05$ ) in soil microbial biomass C and N and enzyme activities at different growth periods under the same water and fertilizer optimal management, and the changes tended to increase first and then decrease with the growth time of *Panax notoginseng*. The soil microbial biomass C and N content ranged from high to low in flowering, fruiting period, seedling period, and rooting period, with the highest in flowering (rainy season) and the lowest in rooting period (dry season). Bargali, K et al. found that soil microbial biomass carbon of different forest types showed significant seasonal changes, with the minimum value in winter and the maximum value in the rainy season (Bargali et al., 2018). Lepcha, N.T. et al. studied the effects of land use types and seasonal changes in the eastern Himalayas on soil microbial biomass carbon and nitrogen and found that soil microbial biomass carbon and nitrogen of different land use types showed strong seasonal differences ( $p < 0.001$ ), with the peak occurring in the rainy season and the lowest in winter (Lepcha et al., 2020). In this study, the highest soil microbial biomass C and N content occurred in the flowering season (rainy season) and the lowest in the root growth period (dry season) because the dry and wet seasons in the study area were distinct, with rainy summer, dry winter and spring, abundant rainfall in summer, humid air, and small evaporation. The soil temperature and humidity were suitable for the growth, reproduction, and decomposition activities of soil microorganisms, and microbial increase promoted the conversion and accumulation of soil microbial biomass C and N content. Huang et al. found that the interaction between increased irrigation amount and season had significant effects on MR, MBC, and MBN but significantly increased in summer and autumn, with no effect in spring and winter. Rehydration in summer and autumn increased bacterial biomass as well as bacterial and fungal diversity and abundance (Huang et al., 2018), which was in contrast to the finding by Li et al. that soil microbial biomass carbon and nitrogen in the Mediterranean agro-ecological system had the lowest value in summer and the highest value in autumn (Li et al., 2014) because of the different climatic types in the two regions. The Mediterranean region was hot and dry in summer and warm and rainy in winter, in contrast to the rainy summer and dry winter and spring in the studied region.

This study found significant differences ( $p < 0.05$ ) between soil microbial biomass C and N and enzyme activities in the same growth period under different water and fertilizer optimal management, and the soil microbial biomass C and N content increased with the increase of irrigation and fertilizer application amount, of which, the W2F4 treatment at the rooting period and the W3F4 treatment at the seedling, flowering and fruiting periods greater than the other treatments in all reproductive stages. The study showed that long-term fertilization improved soil microbial biomass carbon (SMBC) and dehydrogenase activities, and organic fertilizers had a greater effect on SMBC and dehydrogenase activities than mineral fertilizers (Luo et al., 2015). Increases in lower soil elemental and microbial biomass stoichiometry (SMBC:SMBP and SMBN:SMBP) and LAP were mainly due to the combined use of manure and mineral fertilizers accelerating SOC and N mineralization (Yang et al., 2015). In this study, the worst treatments for soil microbiomass carbon, nitrogen and enzyme activity were W1F2 and W1F1 for both the rooting and seedling periods, respectively, and W2F1 and W1F1 for both the flowering and fruiting periods, respectively.

#### 3.2 Effect mechanism of water and fertilizer optimal management on soil enzyme activity of *Panax notoginseng*

Soil enzymes were key components for catalytic decomposition of soil nutrient conversion, and their activity in soil could be used as a measure of soil health index (Ashraf et al., 2020; Kotroczo et al., 2014). This study found significant differences in soil urease, sucrase, acid phosphatase, and catalase activities at different growth periods under optimal management of that same water and fertilizer ( $p < 0.05$ ), and the increasing and then decreasing trends were observed with the prolongation of growth period of *Panax notoginseng*, which ranged from large to small in flowering period, fruiting period, seedling period and rooting period. This was consistent with the findings of Peibing et al. that soil enzyme activities had obvious seasonal variation characteristics, with the soil sucrase activity in summer > autumn > spring > winter, and urease activity in summer > spring > autumn > winter. The results showed that the two soil enzyme activities were the highest in summer and the lowest in winter similar (Pei et al., 2018). Zhu et al. believed that soil enzyme activities had obvious seasonal variation characteristics, with the peak values of soil catalase, phosphatase, and urease of reed community appearing in the exuberant growth period, and the trough value appearing in the germination period and the leaf developing period (Zhu et al., 2017). In this study, the flowering period of *Panax notoginseng* was in summer and it grew vigorously, while the root increasing period was in winter, which was the germination period. Therefore, the flowering period of soil enzyme activity of *Panax notoginseng* was the largest, and the root increasing period was the smallest. The reason was that summer was the rainy season, with plenty of rainfall. The soil temperature and humidity were suitable for the growth and reproduction of microorganisms, and the enzyme activity in the soil was increased by the increase of microbial activity. This study found that under different water and fertilizer optimal management, soil urease, sucrase, acid phosphatase, and catalase showed significant differences in the same growth period ( $p < 0.05$ ). The contents of soil urease, sucrase, acid phosphatase, and catalase increased with the increase of irrigation amount and fertilizer application amount, and the treatment of soil urease, sucrase, acid phosphatase, and catalase W3F4 at the flowering period was the largest.

This study found a significant correlation between soil microbial biomass C and N and soil enzyme activities ( $p < 0.05$ ), a very significant positive correlation between soil acid phosphatase and catalase and soil microbial biomass C and N ( $P < 0.01$ ), and a significant positive correlation between urease and sucrase and soil microbial biomass C and N ( $P < 0.05$ ). This was similar to the finding by Eivazi, F. et al. that the activities of soil urease, sucrase, and alkaline phosphatase had a very significant positive correlation with the soil microbial biomass of carbon, nitrogen, and phosphorus (Eivazi et al., 1996). Yang et al. found a highly significant positive correlation between soil microbial biomass C, N, and P and soil urease, sucrase, and alkaline phosphatase activities (Yang et al., 2015). The study also reveals that soil invertase, acid phosphatase, and catalase were the main factors affecting the regulation of microbial biomass c and n, while urease was an important factor affecting microbial biomass C and N.

### 3.3 Mechanism of interaction of water and fertilizer on soil microbial biomass C and N and enzyme activity of *Panax notoginseng* in farmland

Vepsilinen et al. suggested that chemical fertilizers affected soil enzyme activity by improving soil physical and chemical properties and microflora and that an appropriate ratio of water to fertilizer had an important effect on improving soil fertility and crop yield (Vepsalainen et al., 2001). Reasonable irrigation can improve soil enzyme activity. Too much or too low soil moisture is not conducive to the growth and reproduction of soil microorganisms, resulting in the reduction of soil enzyme activity. This study found different distribution characteristics of soil microbiomass carbon, nitrogen and enzyme activity optimal scheme fit under different optimal water and fertilizer management at different fertility periods. The Ci values under the same irrigation regime were F4, F3, F2, and F1 in descending order, and the benefits and drawbacks of the microbial biomass carbon, nitrogen, and enzyme activity of *Panax notoginseng* soil were F4, F3, F2, and F1 in descending order.; Under the same fertilization treatment, the Ci values decreased and then increased with the increase of irrigation, and the advantages and disadvantages of microbial biomass carbon, nitrogen and enzyme activity of *Panax notoginseng* soil were W2, W1 and W3 in descending order. The Ci value of the W2F4 treatment was the largest at 0.836 during the rooting period, followed by the Ci value of the W3F4 treatment at 0.794, and the worst treatments for soil microbial biomass carbon, nitrogen and enzyme activity were W1F2 and W1F1 with Ci values of 0.214 and 0.133, respectively. The maximum Ci value was 0.806 for the

W3F4 treatment at the seedling period, followed by 0.801 for the W2F4 treatment, and the worst treatments for soil microbial biomass carbon, nitrogen and enzyme activity were W1F2 and W1F1 with Ci values of 0.263 and 0.111, respectively. The maximum Ci value for the W3F4 treatment was 0.733, followed by 0.568 for the W2F3 treatment, and the worst Ci values for soil microbiomass carbon and nitrogen and enzyme activity were 0.216 and 0.189 for the W2F1 and W1F1 treatments, respectively. The maximum Ci value for the W3F4 treatment was 0.835, followed by 0.766 for the W3F3 treatment. The worst soil microbial carbon, nitrogen and enzyme activity treatments were W2F1 and W1F1, with Ci values of 0.273 and 0.068, respectively.

## 4 Conclusion

The characteristics of soil microbial biomass C and N and enzyme activities of *Panax notoginseng* in farmland were studied through optimal management of water and fertilizer. The main conclusions were as follows:

- (1) Under the same optimal management of water and fertilizer, the differences in soil microbial biomass C and N content and soil enzyme activity were significant with different growth periods ( $p < 0.05$ ). The soil microbial biomass C and N content and soil enzyme activity increased firstly and then decreased with time, and gradually increased from rooting period to flowering period; There were significant differences in soil microbial biomass C and N content and soil enzyme activity between the same growth period and different water and fertilizer optimal management ( $p < 0.05$ ), and soil microbial biomass C and N content and soil enzyme activity increased with the increase of irrigation amount and fertilizer application amount.
- (2) The correlation coefficients of acid phosphatase and catalase with soil microbial biomass C and N were in the range of 0.90~1.00 under optimal water and fertilizer management, while those of urease and sucrase with soil microbial biomass C and N were in the range of 0.80~0.95. The C and N content of soil microbial biomass influenced soil enzyme activity.
- (3) The comprehensive evaluation of soil microbial biomass C and N, and enzyme activities under optimal water and fertilizer management showed that the W2F4 treatment of *Panax notoginseng* at rooting period had the highest Ci value of 0.836 and the W3F4 treatments at seedling, flowering, and fruiting periods had the highest Ci values of 0.806, 0.733 and 0.835, respectively. The W2F4 treatment at the rooting period and the W3F4 treatment at the seedling, flowering, and fruiting periods were significantly effective in regulating soil microbial biomass C and N, and enzyme activities. That were beneficial to the growth and yield quality improvement of *Panax notoginseng* plants.

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