

Original Research

# Effects of Gaps on Soil Nutrients and Soil Microbial Carbon in a *Pinus massoniana* Forest, Southwestern China

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## Abstract

To study the influences of gap size on the variation in soil nutrients, soil microbial carbon (micro-C), soil samples from *Pinus massoniana* forests were collected in and around different forest gaps with size ranging from 40.38 to 1321.40 m<sup>2</sup> in Guizhou Province. The soil nutrients (include SOC, TP, TK, TKN, valid-P, valid-K and hydro-N) and micro-C in soil samples were analyzed, and the artificial neural networks was employed to explore the importance of different soil nutrients and gap size concerning micro-C. The results indicate that the micro-C<sub>gap</sub> ranged from 38.00 to 660.32 mg kg<sup>-1</sup> with a mean value of 221.24 mg kg<sup>-1</sup>, and the micro-C<sub>forest</sub> ranged from 110.70 to 685.62 mg kg<sup>-1</sup> with a mean value of 356.16 mg kg<sup>-1</sup>. The micro-C<sub>gap</sub>/micro-C<sub>forest</sub> values increased with the increase of gap size in the range from 40 to 600 m<sup>2</sup>, and this trend inversed when gap size was greater than 600 m<sup>2</sup>. Micro-C was mainly associated with valid-P for both gaps and surrounding forests, large gap may lead to the loss of soil N. The results suggested that: (a) SOC, TK, TKN, hydro-N and valid-P are important factors to microbial in forest soils; (b) TP, valid- K, TKN, TK, valid-P and SOC are important factors to microbial in gap soils. In summary, gaps can increase the heterogeneity of soil nutrients and soil microbial, valid-P is a key factor affecting microbial abundance for *Pinus massoniana* forest. These promote the preservation of biodiversity and pedodiversity.

**Keywords:** gap size, soil nutrients, microbial carbon, microbial nitrogen

## Introduction

Gaps are small openings in the forest canopy, and they are naturally caused by the death of few canopy trees or created by mankind [1]. Understanding and predicting the long-term dynamics of forest ecosystems has become an increasing emphasis since the beginning of the last century [2-4]. It was proved that gaps are of great importance to preserve biodiversity and pedodiversity and maintain the complexity of forests structure [5-7]. Since 1969, studies on the impact of forest gaps on forest structure, biomass, and composition have become widely popular among forest ecologists [5, 8-10]. For perfect forest restoration, increasing attention has been laid on designing silvicultural treatments [11]. Creating gaps in the forest canopy is a significant measure in preserving the high species diversity of forests since this event could increase the environmental heterogeneity [2, 6, 12-13]. Gaps may introduce ecological heterogeneity of forest, such as sunlight levels, bio-availability of soil nutrients, litter accumulation, soil pH, decomposition of soil organic matter and regeneration at microsites [7, 14-15]. Previous studies concerning tree-fall gaps have put ever more stress on vegetation dynamics, and limited studies have focused on the the relationship between soil nutrients and soil microbial communities [16-19]. Gap size as a key characteristic of forests has been widely discussed since the 1980s. Some studies claim that gap size change forest environment mainly via the variation of light environments [20-21]. These studies suggested that regeneration of shade-intolerant species is associated with gap size. Once gap is formed, new species will immigrate in this gap. Shade-intolerant species is usually established in larger gaps, while shade-tolerant species usually dominate vegetation in smaller gaps [22].

*Pinus massoniana* Lamb is an important timber species in southern China, and it was characterized with wide distribution, rapid growth, high yield and high comprehensive utilization [23-24]. *Pinus massoniana* plays an important role in forestry development, ecological construction and the realization of forestry double-growth goals [25-27]. However, *Pinus massoniana* plantations in large areas are facing many ecological security problems, such as soil acidification, frequent occurrence of diseases and insect pests, and decreased above- and belowground biodiversity [25, 28]. It has been shown that strengthening appropriate management and regulation of *Pinus massoniana* plantations is an urgent scientific issue to ensure sustainable management of *Pinus massoniana* plantations. Forest canopy gap is a method to improve the structure of forest ecosystem and promote regeneration and succession of forest ecosystem [29-30]. Consequently, researchers have studied a lot of researches on canopy gap of Pine masson. Hitherto, rare studies focus on change of soil nutrients and microbial carbon related to *Pinus massoniana* forest

gaps. In addition, artificial neural network was used in environmental research fields widely, it is a kind of operation model and a large-scale information processing system with neurons as the basic structural unit [31]. After continuous training, a predictive model was established to determine the concentration of atmospheric pollutants [32], predict tropospheric ozone concentration based on meteorological conditions and various air quality parameters [33], measure the net ecosystem production of a Pacific northwest Douglas-fir stand [34], and predict the hydrological response after forest wildfires and soil treatment [35]. Artificial neural network is an efficient and rapid mathematical method for modeling and processing basic models [36].

In summary, soil properties were impacted by multiple factors [37]. Forest gaps were the important factor influencing the soil nutrients and microbial. What is the status of soil nutrients and microbial characteristics under the masson pine canopy, how does the soil nutrients affect microbial carbon, nitrogen and phosphorus under the masson pine canopy? How to determine the gaps size that can promote soil nutrients and biodiversity, these issues are fully worthy of forests management. The artificial neural network is a good way to find the optimization condition. Therefore it is used to to explore the influence of forest gap size on changes in soil nutrients and the soil microbial community, to find the right size gap that improves soil quality and biodiversity and to present valuable information under the implicating of artificial gaps in *Pinus massoniana* forest cultivation.

## Materials and Methods

### Study Area

The research site was in *Pinus massoniana* forest in Longli County (106°56'34"~106°59'34"E, 26°27'21"~26°29'14"N), in Guizhou Province in southwest China. It has a subtropical humid monsoon climate. The average annual temperature and precipitation are 14.8°C and 1,100.2 mm, respectively. The altitude ranges from 1,114 m to 1,235 m, with a mean value of 1,173 m. Coniferous and broad-leaved mixed forest and evergreen broad-leaved forest are main ecosystem types in study area. The main plant species include *Pinus massoniana*, *Camellia oleifera* Abel., *Rosa cymosa* Tratt., *Corylus heterophylla* Fisch. var. *sutchuenensis* Franch., *Quercus fabri* Hance, *Pyracantha fortuneana* (Maxim.) Li, *Myrica rubra* (Lour.) S. et Zucc., *Dicranopteris dichotoma* (Thunb.) Berhn., *Hypericum monogynum* L., *Phragmites communis* (Cav.) Trin. ex Steud., *Rubus corchorifolius* L. F., *Cibotium barometz* (L.) J. Sm., and *Cunninghamia lanceolata* (Lamb.) Hook.

Table 1. Geographic information of the studied sites.

Sampling site	Longitude	Latitude	Gap size (m <sup>2</sup> )	Altitude (m)	Slope aspect	Slope position	Slope gradient (°)
1	106°58'50"	26°29'14"	40.38	1146	East south	Back slopes	10
2	106°56'39"	26°28'13"	121.46	1206	East south	Back slopes	5
3	106°58'41"	26°29'12"	130.00	1143	South	Back slopes	10
4	106°57'7"	26°27'28"	130.94	1152	West south	Back slopes	10
5	106°57'7"	26°27'29"	163.62	1157	West south	Back slopes	10
6	106°56'34"	26°28'18"	192.21	1235	East south	Shoulder slopes	5
7	106°57'1"	26°27'30"	211.47	1169	East south	Shoulder slopes	5
8	106°59'56"	26°29'13"	239.00	1208	South	Back slopes	10
9	106°56'35"	26°28'16"	612.47	1229	East south	Shoulder slopes	5
10	106°57'17"	26°27'21"	919.53	1114	East north	Back slopes	5
11	106°57'3"	26°27'26"	1096.90	1151	East south	Back slopes	6
12	106°57'5"	26°27'25"	1213.65	1147	East south	Foot slopes	5
13	106°56'39"	26°28'12"	1321.40	1198	East south	Back slopes	5

### Soil Sampling and Field Investigation

In consideration of gap size, 13 gaps were selected in this study. As shown in Table 1, the size of the selected gaps ranged from 40.38 to 1321.40 m<sup>2</sup>, and the slope gradients ranged from 5° to 10°. From March to May 2019, sampling was conducted at the selected gaps. In each gap, soils were collected using a steel core sampler (7.0 cm inner diameter) based on a five-

point sampling method, and the mixed soil was used as the final soil sample of the gap (soil<sub>gap</sub>) [13, 38]. At the same time, a paired soil sample (soil<sub>forest</sub>) was collected in the forest (around the gap) approximately 8 m away from the edge of the gap (in the northern, southern, western and eastern aspects of the gap) (Fig. 1). On site, each soil sample was sieved through a 2 mm mesh screen and divided into two sub-samples. One sub-sample was used to determine microbial C, soil

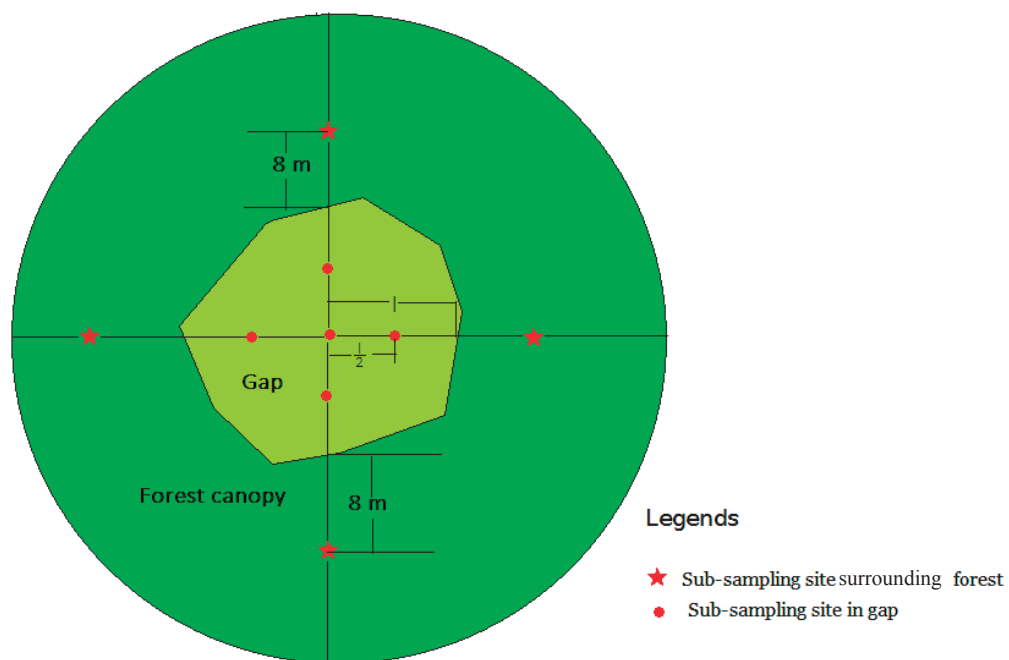


Fig. 1. Sampling design for each gap (soil<sub>gap</sub> was obtained from the mixture of the five soils collected in the gap, and soil<sub>forest</sub> was obtained from the mixture of the four soils collected in the forest canopy).

moisture, valid-P, valid-K and hydro-N; the other sub-sample was air-dried at room temperature in the laboratory for the determination of soil organic carbon (SOC), total potassium (TK), total phosphorus (TP) and total Kjeldahl-N (TKN). The soil samples were put in sterilized polythene bags and transported to the laboratory. Simultaneously, information about sampling sites, including longitude, latitude, gap size, slope aspect, slope position and slope gradient was recorded in the field.

### Analytical Methods and Statistical Analysis

The concentration of TKN was analysed using the Kjeldahl digestion method [14]. The P concentrations were determined using a spectrophotometric technique [39-41]. The concentration of K was analysed using atomic absorption spectroscopy (AAS, ZEEnit 700, from Germany). Soil organic carbon (SOC) content was tested by  $K_2Cr_2O_7$  oxidation at 170-180°C followed by titration with  $FeSO_4$  [42]. Microbial C was analysed according the book "Method and Application of Soil Microbial Biomass Measurement" [43]. Sulfuric acid ( $H_2SO_4$ ), nitric acid ( $HNO_3$ ) and hydrochloric acid (HCl) were guaranteed grade, and the other reagents used were analytical grade. For analytical quality assurance, re-analysis of 10% of the samples was

performed to control the analytical quality, and the uncertainty of the analytical procedure was within 10%.

### Redundancy Analysis

Redundancy analysis was conducted using CANOCO 4.5.1 software (Microcomputer Power, Ithaca, USA). Redundancy analysis allows studying the relationship between two variables. It is a non-symmetric analysis method [44]. In redundancy analysis, the components extracted from A (a variable) are such that they are correlated as much as possible with the variables of B (another variable). Then, the components of B are extracted so that they are correlated as much as possible with the components extracted from A. In this study, soil nutrients were arranged as factors in redundancy analysis to explore their effects on micro-C.

### Prediction of Microbial Abundance with Artificial Neural Networks

To reveal the importance of different factors to microbial abundance, artificial neural networks (ANNs) were applied to predict microbial abundance based on soil nutrients. ANNs is an artificial intelligence method derived from the operation of biological neurons, it via soft computing similar to fuzzy logic, and this

Table 2. Network Information.

Layers	Items		Description
Input Layer	Covariates	1	TKN
		2	Hydro-N
		3	TP
		4	Valid-P
		5	TK
		6	Valid -K
		7	SOC
		8	Gap Size
	Number of Units <sup>a</sup>		8
	Rescaling Method for Covariates		Standardized
Hidden Layer(s)	Number of Hidden Layers		1
	Number of Units in Hidden Layer 1 <sup>a</sup>		2
	Activation Function		Hyperbolic Tangent
Output Layer	Dependent Variables	1	Microbial C
	Number of Units		1
	Rescaling Method for Scale Dependents		Standardized
	Activation Function		Identity
	Error Function		Sum of Squares

a. Excluding the bias unit

approach can solve multivariate nonlinear problems with a suitable amount of data and an appropriate training algorithm [45-47]. In the present study, SOC, TP, TK, TKN, valid-P, valid-K, hydro-N and gap size were defined as factors (covariates), and microbial C was defined as a dependent variable. The network information is listed in Table 2.

## Results and Discussions

### Soil Nutrients, Microbial C and Microbial N in Soils from Different Gaps

As presented in Tables 3 and 4, the total Kjeldahl-N in gap soils ( $TKN_{gap}$ ) ranged from 0.86 g kg<sup>-1</sup> to 3.09 g kg<sup>-1</sup> with a mean of 1.57 g kg<sup>-1</sup>, while the total Kjeldahl-N in forest soils ( $TKN_{forest}$ ) ranged from 0.75 g kg<sup>-1</sup> to 2.50 g kg<sup>-1</sup> with a mean of 1.68 g kg<sup>-1</sup>. The hydro-N in gap soils ( $hydro-N_{gap}$ ) ranged from 82.37 mg kg<sup>-1</sup> to 434.15 mg kg<sup>-1</sup> with a mean of 163.63 mg kg<sup>-1</sup>, and the hydro-N in forest soils ( $hydro-N_{forest}$ ) ranged from 100.65 mg kg<sup>-1</sup> to 198.33 mg kg<sup>-1</sup> with a mean of 135.09 mg kg<sup>-1</sup>. Obviously, the mean of  $TKN_{gap}$  was slightly lower than that of  $TKN_{forest}$ , and the  $hydro-N_{gap}$  was greater than that of  $hydro-N_{forest}$ . In addition, the variances of  $TKN_{gap}$  and  $hydro-N_{gap}$

were much greater (>50%) than those of  $TKN_{forest}$  and  $hydro-N_{forest}$ , respectively.

The TP in gap soils ( $TP_{gap}$ ) ranged from 0.22 g kg<sup>-1</sup> to 0.55 g kg<sup>-1</sup> with a mean of 0.38 g kg<sup>-1</sup>, while the TP in forest soils ( $TP_{forest}$ ) ranged from 0.21 g kg<sup>-1</sup> to 0.59 g kg<sup>-1</sup> with a mean of 0.37 g kg<sup>-1</sup>. The valid-P in gap soils ( $valid-P_{gap}$ ) ranged from 4.01 mg kg<sup>-1</sup> to 21.65 mg kg<sup>-1</sup> with a mean of 13.27 mg kg<sup>-1</sup>, and the valid-P in forest soils ( $valid-P_{forest}$ ) ranged from 4.86 mg kg<sup>-1</sup> to 19.97 mg kg<sup>-1</sup> with a mean of 12.28 mg kg<sup>-1</sup>. In comparison with TKN and hydro-N, there was little discrepancy in the range and variance between  $TP_{gap}$  and  $TP_{forest}$  and between  $valid-P_{gap}$  and  $valid-P_{forest}$ .

The TK in the soils from gaps ( $TK_{gap}$ ) ranged from 7.09 g kg<sup>-1</sup> to 32.69 g kg<sup>-1</sup> with a mean of 13.94 g kg<sup>-1</sup>, while the TK in forest soils ( $TK_{forest}$ ) ranged from 6.25 g kg<sup>-1</sup> to 33.67 g kg<sup>-1</sup> with a mean of 16.65 g kg<sup>-1</sup>. The valid-K in gap soils ( $valid-K_{gap}$ ) ranged from 12.77 mg kg<sup>-1</sup> to 133.55 mg kg<sup>-1</sup> with a mean of 44.88 mg kg<sup>-1</sup>, and the valid-K in forest soils ( $valid-K_{forest}$ ) ranged from 13.67 mg kg<sup>-1</sup> to 71.74 mg kg<sup>-1</sup> with a mean of 37.39 mg kg<sup>-1</sup>. Clearly, these results were similar to those of TP and valid-P. There was little discrepancy in the range and variance between  $TK_{gap}$  and  $TK_{forest}$  and between  $valid-K_{gap}$  and  $valid-K_{forest}$  in comparison with that between TN and hydro-N.

Table 3. Soil nutrients, SOC, microbial C and microbial N in soil samples from different gaps.

Sampling site	TKN	Hydro-N	TP	Valid-P	TK	Valid-K	SOC	Microbial C	Microbial N
	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(g kg <sup>-1</sup> )
1	1.67	82.37	0.40	16.08	18.33	27.89	15.08	38.00	0.26
2	1.79	255.32	0.25	10.23	7.56	113.55	9.17	167.94	0.38
3	1.30	125.55	0.27	10.00	26.17	12.77	17.25	146.76	0.21
4	0.86	211.37	0.52	10.89	12.59	49.04	16.06	87.76	0.38
5	1.10	129.25	0.47	18.42	32.69	27.78	11.08	52.20	0.24
6	0.99	105.52	0.33	9.42	9.24	27.38	6.03	173.32	0.26
7	1.95	143.33	0.41	15.78	7.69	18.67	12.53	493.98	0.36
8	3.09	434.15	0.32	4.01	27.00	57.89	69.31	257.30	0.60
9	1.46	106.55	0.31	11.40	9.16	41.61	7.05	660.32	0.28
10	1.21	102.65	0.46	21.40	7.76	41.36	17.86	200.22	0.49
11	1.87	162.50	0.38	14.35	8.51	51.08	5.45	109.44	0.29
12	1.62	133.78	0.55	21.65	7.39	26.55	8.50	378.24	0.29
13	1.52	134.80	0.22	8.89	7.09	87.86	8.49	110.70	0.31
Minimum	0.86	82.37	0.22	4.01	7.09	12.77	5.45	38.00	0.21
Maximum	3.09	434.15	0.55	21.65	32.69	113.55	69.31	660.32	0.60
Mean	1.57	163.63	0.38	13.27	13.94	44.88	15.68	221.24	0.33
Std. Error	0.16	26.02	0.03	1.45	2.50	7.91	4.62	51.20	0.03
Variance	0.32	8799.29	0.01	27.25	81.25	812.69	277.74	34078.39	0.01

Table 4. Soil nutrients, SOC, microbial C and microbial N in soil samples from different forests.

Sampling site	TKN	Hydro-N	TP	Valid-P	TK	Valid-K	SOC	microbial C	microbial N
	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(g kg <sup>-1</sup> )	(mg kg <sup>-1</sup> )	(g kg <sup>-1</sup> )
1	1.59	133.37	0.34	9.45	24.36	26.40	19.58	311.32	0.19
2	1.63	146.27	0.23	12.14	7.29	49.17	17.02	351.68	0.42
3	1.58	116.41	0.36	6.27	21.52	13.68	13.71	110.70	0.23
4	0.75	100.65	0.38	15.16	28.39	32.82	13.69	247.62	0.26
5	1.43	135.02	0.44	19.97	7.42	34.06	10.98	645.78	0.31
6	1.97	165.13	0.29	7.46	33.67	50.15	13.38	223.92	0.22
7	1.96	149.97	0.59	15.66	7.94	32.28	17.32	265.02	0.24
8	2.11	137.43	0.26	4.86	25.04	31.69	20.34	342.18	0.32
9	1.45	102.62	0.21	12.32	7.23	34.81	8.88	436.11	0.21
10	1.20	106.76	0.33	17.64	7.40	29.54	10.87	592.95	0.33
11	1.58	141.43	0.37	12.06	32.05	41.61	13.07	166.68	0.19
12	2.10	198.33	0.53	17.45	7.92	38.07	10.85	685.62	0.30
13	2.50	122.75	0.47	9.14	6.25	71.74	10.24	250.48	0.22
Minimum	0.75	100.65	0.21	4.86	6.25	13.68	8.88	110.70	0.19
Maximum	2.50	198.33	0.59	19.97	33.67	71.74	20.34	685.62	0.42
Mean	1.68	135.09	0.37	12.28	16.65	37.39	13.84	356.16	0.26
Std. Error	0.45	27.14	0.11	4.71	10.90	13.97	3.67	183.03	0.07
Variance	0.20	736.75	0.01	22.22	118.80	195.13	13.48	33500.64	0.01

The SOC in gap soils (SOC<sub>gap</sub>) ranged from 5.45 g kg<sup>-1</sup> to 69.31 g kg<sup>-1</sup> with a mean of 15.68 g kg<sup>-1</sup>, while the SOC in forest soils (SOC<sub>forest</sub>) ranged from 8.88 g kg<sup>-1</sup> to 20.34 g kg<sup>-1</sup> with a mean of 13.84 g kg<sup>-1</sup>. This results were similar to those of TKN and hydro-N. There was a large discrepancy in the range and variance between SOC<sub>gap</sub> and SOC<sub>forest</sub>. The variance of SOC<sub>gap</sub> (277.74) was much greater than that of SOC<sub>forest</sub> (13.48). The soil microbial C of gap soils (micro-C<sub>gap</sub>) ranged from 38.00 to 660.32 mg kg<sup>-1</sup>, and the mean value was 221.24 mg kg<sup>-1</sup>. The soil microbial C of forest soils (micro-C<sub>forest</sub>) ranged from 110.70 to 685.62 mg kg<sup>-1</sup>, and the mean value was 356.16 mg kg<sup>-1</sup>. The mean value and variance of micro-C<sub>gap</sub> were greater than those of micro-C<sub>forest</sub>. The soil microbial N values of the gaps (micro-N<sub>gap</sub>) ranged from 0.21 to 0.60 mg kg<sup>-1</sup>, and the mean value was 0.33 mg kg<sup>-1</sup>.

#### Soil Nutrients Characteristics under Different Gap Sizes

It is well known that soils in karst mountainous regions are highly heterogeneous, including soil physical and chemical properties, such as soil texture, soil nutrients, and soil organic/inorganic pollutants. It is inadvisable to study the gap effect on nutrients based on their distribution characteristics. In this study,

the effects of gap size on soil nutrients were studied based on the ratio of their contents in forest soil and gap soil, and the results are shown in Fig. 2. The values of TKN<sub>gap</sub>/TKN<sub>forest</sub> were within the range of 0.50 to 1.46 with a mean value of 0.96, and the values of hydro-N<sub>gap</sub>/hydro-N<sub>forest</sub> were within the range of 0.62 to 3.16 with a mean value of 1.24. For the small gaps (<400 m<sup>2</sup>), both the TKN<sub>gap</sub>/TKN<sub>forest</sub> and hydro-N<sub>gap</sub>/hydro-N<sub>forest</sub> values broadly fluctuated with no obvious trend. For gaps greater than 400 m<sup>2</sup>, the TKN<sub>gap</sub>/TKN<sub>forest</sub> values tended to decrease with increase of gap size. However, the values of hydro-N<sub>gap</sub>/hydro-N<sub>forest</sub> tended to be consistent when gaps were greater than 400 m<sup>2</sup>. The values of TP<sub>gap</sub>/TP<sub>forest</sub> were within the range of 0.47 to 1.48 with a mean value of 1.07, and the values of valid-P<sub>gap</sub>/valid-P<sub>forest</sub> were within the range of 0.72 to 1.70 with a mean value of 1.11. The characteristics of the TP<sub>gap</sub>/TP<sub>forest</sub> values were similar to those of the TKN<sub>gap</sub>/TKN<sub>forest</sub> values. Nevertheless, the characteristics of the valid-P<sub>gap</sub>/valid-P<sub>forest</sub> values were significantly different from the values of hydro-N<sub>gap</sub>/hydro-N<sub>forest</sub>. With an increase in gap size, the value of hydro-N<sub>gap</sub>/hydro-N<sub>forest</sub> first decreased (<400 m<sup>2</sup>), then increased (400 m<sup>2</sup><1000 m<sup>2</sup>), and finally decreased (>1000 m<sup>2</sup>). The values of TK<sub>gap</sub>/TK<sub>forest</sub> and valid-K<sub>gap</sub>/valid-P<sub>forest</sub> presented similar characteristics, and they broadly fluctuated with no trend when the gap size was lower than 400 m<sup>2</sup>.

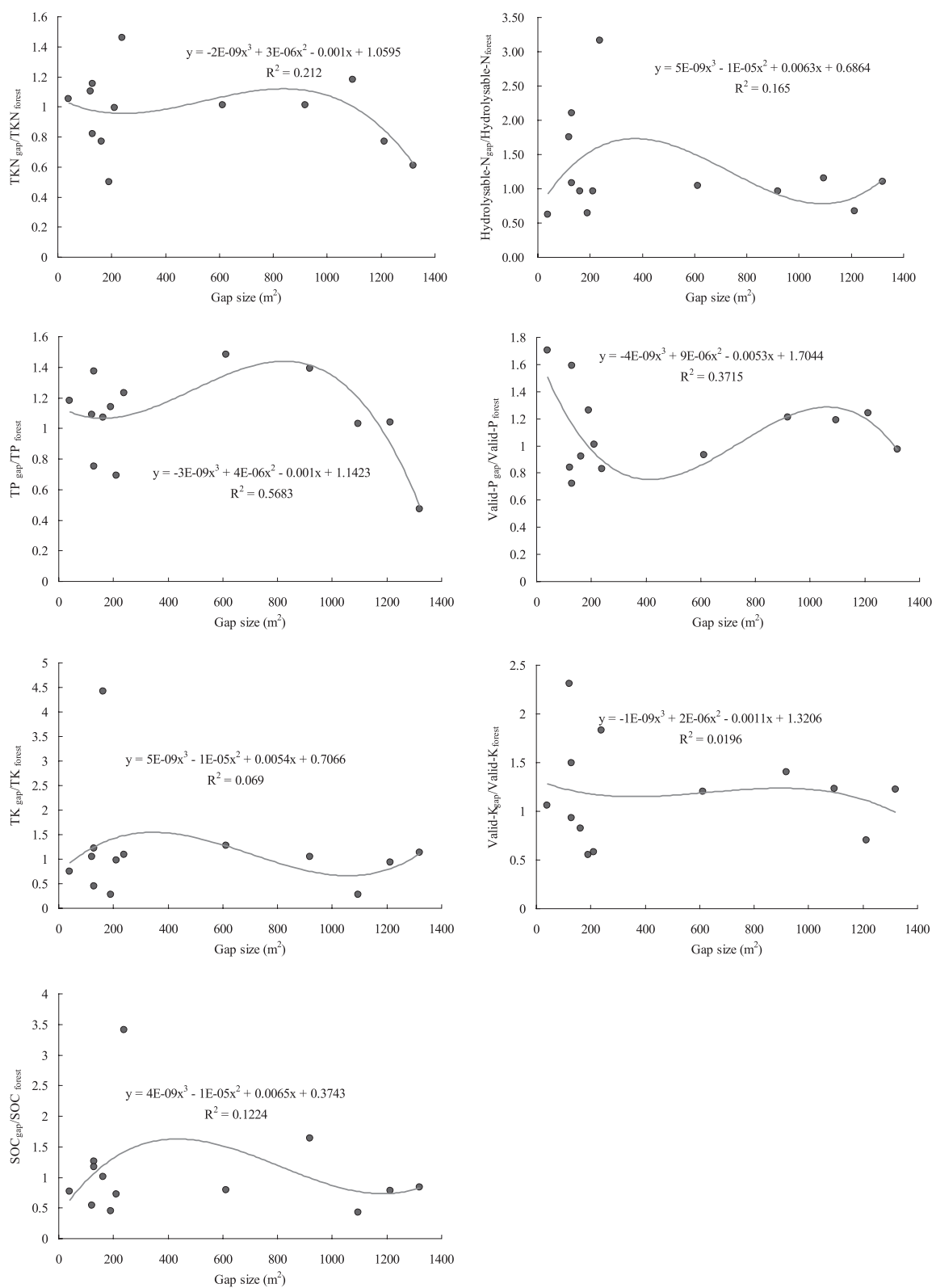


Fig. 2. Variation in nutrient ratio values between the gaps and forests with an increase in gap size.

and tended to be consistent when the gaps were greater than 400 m<sup>2</sup>. The SOC ratio values between the gaps and forest ranged from 0.42 to 3.41 with a mean value of 1.06, and most of these values were lower than 1.

### Characteristics of Micro-C under Different Gap Sizes

The values of micro-C<sub>gaps</sub>/micro-C<sub>forest</sub> were in the range of 0.12 to 1.86 (Fig. 3). Generally, the micro-C<sub>gap</sub>/

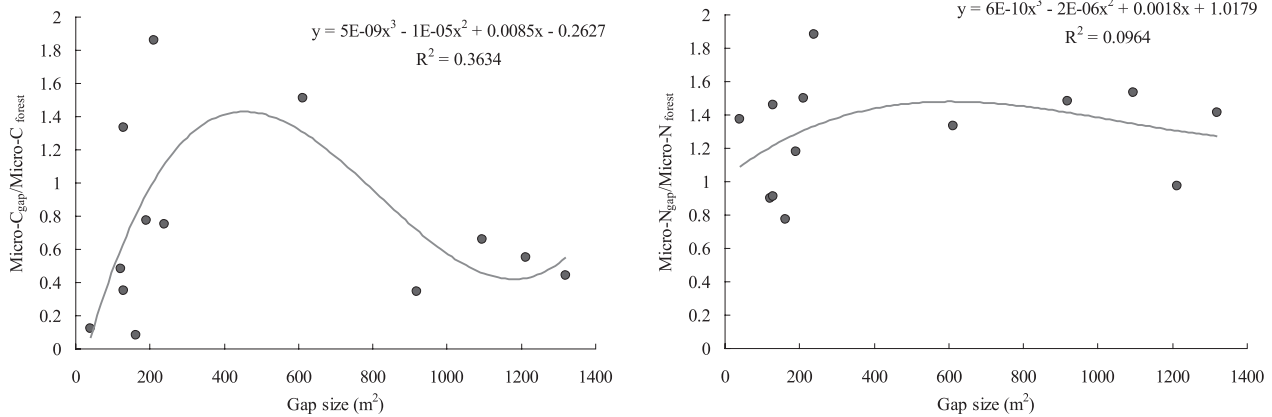


Fig. 3. Characteristics of micro-C<sub>gap</sub>/micro-C<sub>forest</sub> and micro-N<sub>gap</sub>/micro-N<sub>forest</sub> under different gap sizes.

micro-C<sub>forest</sub> value increased with increasing gap size (40~600 m<sup>2</sup>), and it inverted when gaps were greater than 600 m<sup>2</sup>. Clearly, most of the micro-C<sub>gap</sub>/micro-C<sub>forest</sub> values were lower than 1.00. The values of micro-N<sub>gap</sub>/micro-N<sub>forest</sub> were in the range of 0.90 to 1.88. This range was much smaller than that of micro-C<sub>gap</sub>/micro-C<sub>forest</sub>. In addition, most of the values of micro-N<sub>gap</sub>/micro-N<sub>forest</sub> were greater than 1.0. With the increase in gap size, the values of micro-N<sub>gap</sub>/micro-N<sub>forest</sub> fluctuated around approximately 1.2, and the fluctuating amplitude decreased as the gap size was greater than 600 m<sup>2</sup>.

### Relationships among Soil Nutrients, Micro-C and Micro-N

Redundancy analysis (RDA) and Pearson correlation analysis were conducted to explore the relationship among soil nutrients, micro-C and micro-N, and the results were present in Fig. 4. In gap soils, micro-C

was negatively associated with SOC, TP, Hydro-N and valid-K, and positively associated with TKN and valid-P. In forest soils, micro-C was negatively associated with SOC, TK and TKN, and positively associated Hydro-N, valid-K, TP and valid-P. In gap soils, there was no nutrient that presented a significant correlation to micro-C and micro-C/N (Table 5). However, significant correlations were observed between micro-N and TKN ( $r^2 = 0.59, p < 0.05$ ), between micro-N and Hydro-N ( $r^2 = 0.75, p < 0.01$ ), and between micro-N and SOC ( $r^2 = 0.76, p < 0.01$ ). For the soils from the forests, no nutrients presented a significant correlation with micro-N. Micro-C was significantly correlated with valid-P ( $r^2 = 0.71, p < 0.01$ ) and TK ( $r^2 = 0.61, p < 0.05$ ), and micro-C/N was significantly correlated with valid-P ( $r^2 = 0.61, p < 0.05$ ). Interestingly, micro-C/N was significantly correlated with SOC for both gaps ( $r^2 = 0.96, p < 0.01$ ) and forests ( $r^2 = 0.89, p < 0.01$ ). There were also some significant correlations among soil nutrients. For the gap soils, TKN was significantly

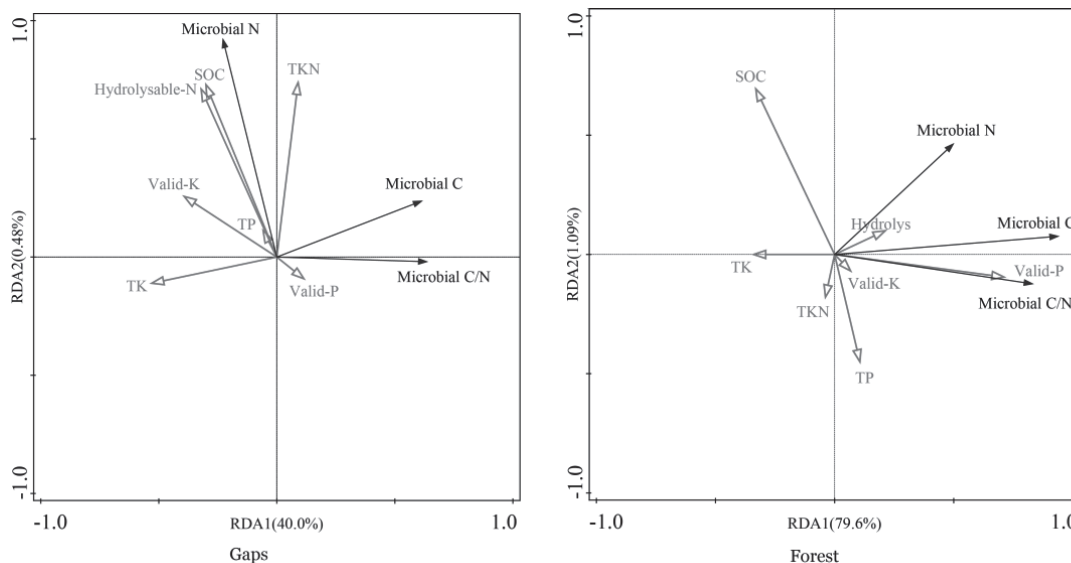


Fig. 4. Redundancy analysis of nutrients effect on micro-C, micro-N and micro-C/N.



Table 5. Pearson relationship among nutrients, Micro-C, Micro- N and Micro- C/N.

	TKN	Hydro-N	TP	Valid-P	TK	Valid-K	SOC	Micro-C	Micro-N	Micro-C/N
TKN		0.73**	-0.27	-0.36	0.15	0.24	0.73**	0.23	0.59*	0.04
Hydro-N	0.57*		-0.18	-0.60*	0.28	0.45	0.83**	-0.03	0.75**	-0.22
TP	0.29	0.38		0.72**	0.01	-0.49	-0.09	-0.01	0.04	-0.04
Valid-P	-0.41	0.08	0.46		-0.18	-0.41	-0.46	0.05	-0.23	0.07
TK	-0.18	0.01	-0.29	-0.53		-0.32	0.51	-0.35	-0.03	-0.33
Valid-K	0.53	0.22	0.07	-0.06	-0.18		0.06	-0.18	0.36	-0.25
SOC	0.11	0.15	-0.13	-0.45	0.38	-0.27		-0.01	0.76**	-0.21
Micro-C	-0.08	0.25	0.14	0.71**	-0.61*	-0.05	-0.37		0.10	0.96**
Micro-N	-0.09	0.15	-0.18	0.30	-0.43	0.03	0.14	0.50		-0.17
Micro-C/N	-0.04	0.17	0.15	0.61*	-0.52	-0.048	-0.438	0.89**	0.08	

correlated with hydro-N ( $r^2 = 0.73$ ,  $p < 0.01$ ) and SOC ( $r^2 = 0.73$ ,  $p < 0.01$ ); hydro-N was significantly correlated with valid-P ( $r^2 = -0.60$ ,  $p < 0.05$ ) and SOC ( $r^2 = 0.83$ ,  $p < 0.01$ ); and TP was significantly correlated with valid-P ( $r^2 = 0.72$ ,  $p < 0.01$ ). However, only one significant correlation between TKN and hydro-N ( $r^2 = -0.57$ ,  $p < 0.05$ ) was observed among the soil nutrients in the forestland.

#### Prediction of Soil Micro-C with Artificial Neural Network

Artificial neural networks (ANNs) have been used to predict micro-C with SOC, TP, TK, TKN, valid-P, valid-K, hydro-N and gap size as factors. For the gap soils, the correlation coefficients between the observed values and predicted values of micro-C ranged from 0.928 to 0.998, with a mean value of 0.951 (ten repeats); for the forest soils, the correlation coefficients between the observed values and predicted values of micro-C ranged from 0.931 to 0.999, with a mean value of 0.949. These results indicate that this method might be a feasible way to predict micro-C based on soil nutrients (Fig. 5). For the gap soils, the normalized importance of the studied factors occurred in the following order TP>valid-K>TKN>TK>valid-P>SOC>hydro-N>gap size; for the forest soils, the normalized importance of the studied factors occurred in the following the order: SOC>TK>TKN>hydro-N>valid-P>valid-K>TP>gap size.

#### Physiochemical Variation of Gaps along with Gap Size in *Pinus Massoniana* Forest

There was high discrepancy in soil nutrients and micro-C content and spatial distribution between gaps and forest, these results were close to Wang's research [48] which is adjacent to this study area. The distribution regular of different soil nutrient factors were greater differences between gaps and forest as well. The  $TKN_{gap}$

content was lower than  $TKN_{forest}$ , the  $hydro-N_{gap}$  was higher than which in forest, while the variances of TKN and hydro-N in gaps were higher than those in forest. In addition, there were no significant distribution regular of TP and valid-P, TK and valid-K, SOC and micro-C between gaps and forests. These result in the ratios of different soil nutrients ( $X_{gap}/X_{forest}$ ) were lacking in consistency, and its change trend was diverse along the gap size. The  $hydro-N_{gap}/hydro-N_{forest}$  first decreased be lower than 400 m<sup>2</sup> and then increased until 1000 m<sup>2</sup> and then decreased. The 400 m<sup>2</sup> is a critical value for  $TK_{gap}/TK_{forest}$  and  $valid-K_{gap}/valid-P_{forest}$  which presented with no trend when the gap size was lower than 400 m<sup>2</sup> and tended to be consistent when the gaps were greater than 400 m<sup>2</sup>. While the critical value of  $micro-C_{gaps}/micro-C_{forest}$  was 600 m<sup>2</sup>, it's increased with increasing gap size (40~600 m<sup>2</sup>), and it inversed when gaps were greater than 600 m<sup>2</sup>. The complex spatial distribution of soil nutrients between gap and forest, revealed reveal that the gaps size was an important impact factor to control the soil properties.

Circumstance within gap is usually different from closed canopies [49]. There are some reasons for the discrepancies between gaps and surrounding forestland. The most important reason is that the physiochemical conditions, such as soil moisture, sunlight, temperature and available nutrients, were different from each other [13-14]. Gaps are niches which provide ideal environment for rapid plant reproduction and growth [50]. For example, the soil moisture of gap was greater than that of forestland soils. Bartsch suggested that less than 81% of precipitation above the forest canopy reaches the forest ground, and approximately 90% reaches the gap centre [51]. Xu et al. [13] found that in comparison with that in small gaps and adjacent canopy-covered plots, the 0-10 cm soil in large gaps result in a huge increase in the light transmission ratio and air and soil temperatures and a decline in soil moisture, organic matter, N and P. The authors suggested that rapid litter decomposition and increased nutrient

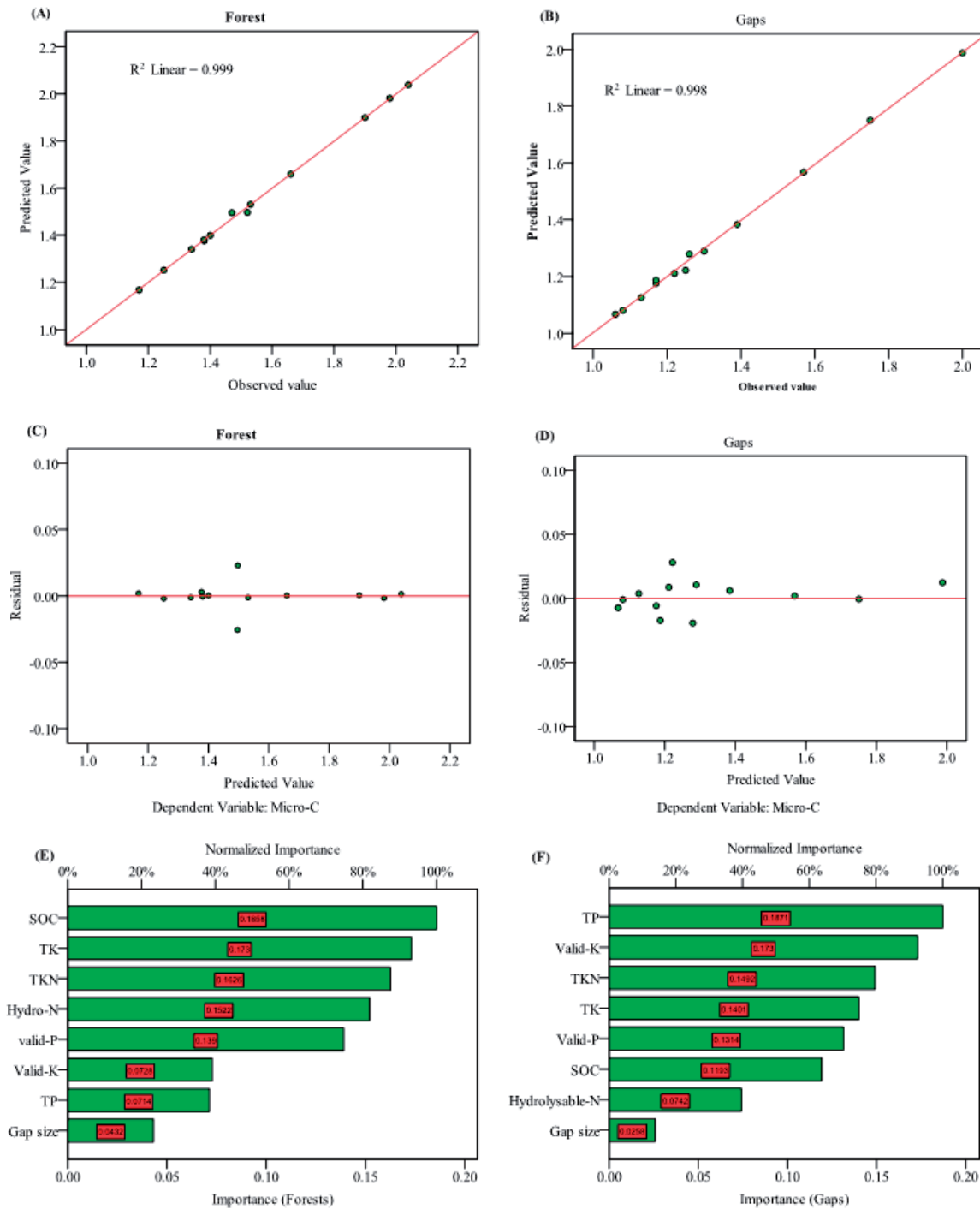


Fig. 5. Prediction of soil micro-C with an artificial neural network based on soil nutrients and gap size.

leaching reduced soil nutrient availability and enzyme activity in the large gaps [13]. In the present study, discrepancies were found between the soil nutrients in the gaps and surrounding forestland in the present study. Given the mean values,  $TKN_{gap}$  and  $TK_{gap}$  were slightly lower than  $TKN_{forest}$  and  $TK_{forest}$ , respectively. The hydro-N, TP, valid-P, valid-K and SOC in the soils from the gaps were slightly greater than those in the soils from the forests. The gap size varies, so does the soil nutrients. For the small gaps (<400 m<sup>2</sup>), the

values of  $TKN_{gap}/TKN_{forest}$ ,  $TP_{gap}/TP_{forest}$  and  $TKN_{gap}/TKN_{forest}$  fluctuated around 1 with a large range. However, all of the values of  $TKN_{gap}/TKN_{forest}$  were close to 1, and the values of  $TP_{gap}/TP_{forest}$  and  $TKN_{gap}/TKN_{forest}$  tended to decrease with increasing gap size once the size was larger than 400 m<sup>2</sup>. Therefore, this research results are different from the previous. In fact, the results from this study suggested that there were no constant variation rules for all nutrients (elements or substances) with the increase in gap size. Nevertheless,

there is some valuable information worth attention. For the smaller gaps (<600 m<sup>2</sup>), all of the ratios between nutrients (TKN, hydro-N, TP, valid-P, TK and valid-K) in the gaps of the surrounding forestland fluctuated with a rather large range and no rule. For the large gaps (>600 m<sup>2</sup>), nutrient variation rules were found.

#### Gap Size and Closure Time in *Pinus Massoniana* Forest

Forest gaps are a special type of ecological system, and plants in gaps usually consist of relatively shorter trees, brush plants, and annual and perennial plants. Gap size significantly affects plant growth, nutrient cycling, micro-environmental characteristics, biological processes, and succession processes. Gap size is also an important parameter determining the regeneration niche of tree species [8, 21, 52, 53]. Small forest gaps will gradually disappear over a shorter time, and large forest gaps will gradually shrink over a longer time [54]. It has been found that a gap with a diameter of 5-15 m will be closed within 5-40 years, while it will take 30-60 years for a gap with an area of 200-300 m<sup>2</sup> to close [55]. In addition, gap size is also a key factor governing the structure of plant communities [5, 15]. Based on the field investigation, the small gaps usually disappeared within 3 to 6 years. Small gaps usually featured sufficient light and heat, relatively fewer filling plants, more seedlings in the centre and edge, and less competition among edge trees (*Pinus massoniana*). Therefore, vigorous growth of the canopy led to the closure of the gap within a short time. In the large gaps, the seeds of *Pinus massoniana* have a difficult time falling to the soil since the gaps are usually filled with *Dicranopteris pedata* and some shrubs. Even if the seeds of *Pinus massoniana* fell into the soils and sprouted, it had to compete for environmental resources with *Dicranopteris pedata* and shrubs. Consequently, *Pinus massoniana* seedlings grow very slowly and even die due to weak competition in comparison with other gap plants. Thus, control of the gap size may be a feasible choice in the regulation of forests to satisfy the aim of silviculture. To preserve biodiversity and pedodiversity, gap sizes should be larger than 400 m<sup>2</sup> and smaller than 600 m<sup>2</sup> for *Pinus massoniana* forests in karst mountainous areas. To increase timber, however, efforts should be focused on the restoration of gaps in areas larger than 400 m<sup>2</sup> since they cannot close naturally in a short period.

#### Importance of Factors in Prediction of Soil Micro-C with Artificial Neural Network

As an approach derived from the operation of biological neurons, ANNs can solve some multivariate non-linear problems [33, 56, 57]. Results from present study indicated that the correlation coefficients between observed values and predicted values were greater than 0.90. Therefore, it is a possible way to predict micro-C

with selected factors. It is found that the importance of the same factor differs between gaps and forest in prediction of soil micro-C. For instance, the importance value of SOC was up to 0.1858 in prediction micro-C in forests, and it was only 0.1193 in prediction micro-C in gaps. We believed that decomposition of plant residues in gaps is more rapid than that in forest. Ni et al. reported that the forest gap had important influence on early litter humification, and considered that forest gap reduces the accumulation rate of soil organic matter [58]. It is believed that the forest soils were covered with thick layer of tree leaves and twigs creating an oxygen free environment which is not conducive to microbial growth. On the contrary, TP plays the most important role in prediction of micro-C in gap soils, and its importance value was up to 0.1871. In the prediction of soil micro-C in both gaps and forests, gap size presented little importance. For soil microbial community, gap size is not a direct affecting factor. Gap size does not directly affect microbial community structure and abundance. Its influence on microbial community structure is mainly realized by affecting the physical and chemical properties of soils, nutrient content and so on [59]. Therefore, gap size is not so important in the prediction of soil micro-c as the other factor for both gaps and forests.

#### Soil Microbe and Future Research

Soil microbe is an important component of the soil ecosystem, which can regulate ecosystem functions and effect important ecological processes related to soil organic matter conversion and biogeochemical cycles [60]. Moreover, its metabolic activities play a leading role in ecological processes such as soil carbon and nitrogen cycling and retention [61, 62]. Based on field investigation and laboratory analysis, the study has shown that gaps lead to variation in soil nutrients and SOC from surrounding forest soils, consequently leading to variation in microbial abundance. Valid-P was significantly correlated with micro-C ( $r = 0.71$ ,  $p < 0.01$ ) and micro-C/N ( $r^2 = 0.61$ ,  $p < 0.05$ ). In recent years, researchers have studied the relationship among soil micro-C, soil micro-N and soil micro-P under the natural or artificial forest gap [13, 37, 62, 63]. There are limited studies that has deeply analyzed the response of soil microbe to size and developmental stages of forest gap and revealed the effects of forest gap on soil microbe. Therefore, in ongoing research, the author has monitored the effects of soil hydrological factors under different size and different development stages in natural forest gap on soil microbial community structure and soil microbial diversity in long-term. The author attempts to analyze genetic diversity of soil microbial diversity and functional genes to forest gap. These researches mentioned above will let researchers to understand how soil microbe affect soil respiration, soil carbon fixation, and soil carbon-nitrogen cycle,

so as to provide theoretical basis for global carbon cycle research. In addition, the soil nutrients are the base of ecosystem services, therefore the forests ecosystem services capacity coupling with the change of gaps size should be focused more in the future.

### Conclusions

Gaps lead to variation of soil nutrients and SOC from surrounding forest soils, consequently leading to variation in microbial abundance. Small gaps (<400 m<sup>2</sup>) lead irregular changes of all studied soil nutrients. Micro-C was significantly correlated with valid-P ( $r = 0.71$ ,  $p < 0.01$ ) in forest soils. It is considered that valid-P is a key index affecting microbial abundance for canopy forest. Micro-N was significantly correlated with TKN and Hydro-N, and these would be important factor affecting microbial in gap soils. The forest edge effect became weak with the increase in gap size, and this effect continued to differ with gap size for different nutrients. During the *Pinus massoniana* forest cultivation, it is advisable to control the gap size based on the cultivation aim. In addition, soil micro-C was mainly associated with soil nutrients, and gap size showed no direct effect on the abundance of soil micro-C.

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### Conflict of Interest

The authors declare no conflict of interest. We certify that the submission is not under review at any other publication.

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