EFFECTS OF SOIL MICROORGANISMS ON BAMBOO (*Phyllostachys praecon* C. D. Chu et C. S. Chao *'Prevernalis)* GROWTH

by

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ABSTRACT

Key words: Bacteria, fungi, seasonal characteristics, Soil microorganisms, *Phyllostachys praecon* C. D. Chu et C. S. Chao *'Prevernalis*.

Soil microorganisms and plants together form a stable dynamic system in which they interact and influence each other. The growth and development of plants provide nutrients to the soil microorganisms, which in turn promote and contribute to the growth of plants. In addition, microorganisms are the most active components of the soil. They are diverse and numerous, and each performs a unique function in the soil ecosystem. Different soil environments will develop different microbial population structures. Therefore, in this paper, I will verify the relationship between soil microorganisms and temperature by measuring and analyzing the microbial biomass at specific locations for a stand of bamboo *Phyllostachys praecon*.

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1.0. INTRODUCTION

Soil is a dynamic system and there are many biotic and abiotic organisms there which form a large network (Alown *et al.,* 2021). The impact of these interactions simultaneously gives the soil a very important place in the overall natural environment. In general, the reason for the vitality of soils has always been closely linked to the diversity of soil microorganisms and the interactions of different microorganisms.

The diversity of microorganisms and their interactions are important in the various mass and energy exchanges that occur between various substances in the whole ecosystem. Microorganisms are ubiquitous in soils and influence soil properties (Jia *et al.,* 2020)

The central question posed by the link between microbial diversity and soil function is to understand the relationship between genetic diversity and community structure and between community structure and function (Nannipieri *et al.,* 2003). A richer and more diverse soil microbial community is an important factor in maintaining the stability of terrestrial ecosystems.

Often, soil microbial communities are affected by the magnitude of natural fluctuations (*e.g.,* temperature, plant growth, or rainfall; Michele *et al.,* 2011). Soil diversity can be easily influenced by the surrounding environment and this paper investigates the relationship between soil microbial diversity and seasonal changes in temperature.

1.1. Objective and Hypothesis

When studying plant growth, the variation of soil microorganism content needs to be well grasped (Richard *et al.,* 2017). In the natural environment, constant changes in temperature can have a great impact on the content of soil microorganisms. Therefore, the hypothesis of this study is that as the temperature increases, there is a significant increase in the microbial population of the soil. An article by Luo and her team (2016) is a study of the relationship between the content of soil microorganisms and temperature variation and will be examined for this thesis. The purpose of this study is to investigate the different associations between the microbial content of the soil and temperature.

Phyllostachys violascens (*Phyllostachys praecox* C. D. Chu et C. S. Chao *'Prevernalis'*) is a species of bamboo of the genus *Phyllostachys* in the subfamily *Bambusoideae* of the family *Poaceae*, native to the hilly plains of northwest Zhejiang. It has been widely introduced in various places (Chen 2005). It is a dual-purpose economic bamboo species. Some production practices such as continuous mowing, loosening, and digging of bamboo shoots have led to soil erosion and reduced productivity (Tong *et al.,* 2020). The use of organic mulch to increase soil temperature to promote early emergence during the management of Lei bamboo resulted in a large short-term increase in soil organic matter (Zhao *et al.,* 2015).

Temperature can also affect the structure and abundance of soil microorganisms. Thus, my hypothesis is that temperature has an effect on the growth of the *Phyllostachys violascens*, and that as the temperature increases the number of soil microorganisms increases and the growth of the *Phyllostachys violascens* is promoted.

My hypothesis is that the number of microorganisms in the soil will gradually increase as the temperature continues to increase in the *Phyllostachys parecon* plantation.

2.0. LITERATURE REVIEW

2.1. Soil

Chongyang County is located in the southeast of Hubei Province, with more hills and mountains. The topography of the whole county is high in the south and low in the north, gentle in the middle, surrounded by mountains on all sides: it is a mountainous area with many hills and little flat land. The rainfall in Chongyang County is very abundant, with 3.122 billion m^3 of precipitation in a year, making it a water-rich county.

The data on soil microbial content were obtained from a study of soil microorganisms in the Chongyang County area of China conducted by Zishan Luo and her team in 2014. The study area is from 29°12′ to 29°41′ in Chongyang County. This county has a subtropical monsoon climate and four distinct seasons.

Figure 1. The location of Chongyang County.

Source: Google Map Data

The soil types in Chongyang County are rich and diverse, suitable for vegetation growth and agriculture and forestry farming (Tang *et al.,* 2016). Land resources can be used for forestry, agriculture, specialties, aquaculture, etc. and the advantages are very prominent.

The total land area of the county is 195,983 hm^2 , and due to its complex topography, the distribution of land resources varies significantly. Table 1 shows the area and proportion of various types of land. The surrounding mountains and woodlands are predominant, with an area of 10,045 hm^2, accounting for 51.26% of the total land area of the county, and topping the list of land for various industries. The central part of the county is low, with dry land in the hills and mountains and paddy fields in the low-lying flat areas accounting for 19.27% of the total area. In addition, 21.23% is unused land, which is the potential land resource of the county (Lv and Yang 2002).

Table 1. All kinds of land composition in Chongyang County (Tang *et al.,* 2016).

	Arable land	Garden	Woodland	Land for traffic	Residential industrial and mining land	Waters	Water conservancy facilities	Unused land
Area hm^2	3775	2536	100454	797	505	6477	1302	41607
Proportion %	19.27	1.29	51.62	0.41	2.58	3.3	0.66	21.23

Soil and ecosystem processes are constantly changing, and such changes can be found by studying the differences in taxonomic and functional diversity among microbial communities. A better understanding of the microbial community composition and its determinants may deepen our understanding of biogeochemical processes (Drenovsky *et al.,* 2003). So in order to have a better understanding of soil quality, it is first necessary to have some knowledge of the soil, the structure of microorganisms, and their interactions with each other.

Soil is the skin of the earth, the loose layer on the land surface where plants can grow (Zhu *et al.,* 2015). Soil consists of various minerals, organic matter, water, air and microorganisms, etc. It is formed during the evolution of the earth as a natural reference under the combined action of natural factors and human activities (Chen 2010). It is the most basic natural resource on which human beings depend for their survival. Therefore, people cannot live their daily lives without soil and the living organisms that inhabit the soil.

Soil is the largest reservoir of biodiversity on earth, and microorganisms are the main expression of soil biodiversity (Cao 2020). According to the definition of soil, its essential property is its ability to provide nutrients to plants (*i.e.,* plant nutrient functions) and soil microorganisms play a key role in the process of providing these nutrients to plants (Shen and Zhao 2015).

Soil is a structured, heterogeneous and discontinuous system. In general, good soil structure can significantly increase soil microbial activity. Determining the exact community composition in soils is difficult (Nannipieri *et al.,* 2003) and is the source of much soil research. Soil also has social, ecological, economic, cultural and spiritual dimensions of value (Robinson *et al.,* 2012). Therefore, the overall functions and values of soil have a centralized and important place in society.

In soils with high maturation and good fertility, the number of soil microorganisms is higher and the percentage of bacteria is higher (Zhou and Ding 2007). Soil microorganisms are also important for the development of a healthy soil structure.

Through the production of large amounts of cohesive material by soil microorganisms, cemented soil masses are able to aggregate better. Fungal hyphae are also able to make the soil structure more stable. Their mycelium is a filamentous structure that unfolds in the soil and is able to aggregate in a network (Christopher 2017).

Soils have many beneficial soil microorganisms such as bacteria and fungi that help maintain soil and plant health (Ameen *et al.,* 2021) Because bacteria and fungi are the main microorganisms that occur in soils, their reaction with the soil has a great impact on plant growth.

2.2. Soil Microorganisms

Microorganisms in soil include archaea, bacteria, actinomycetes, fungi, algae, and protozoa (Susan 2021). Bacteria are of different shapes, spherical, rod-shaped and spiral. Individual cells are generally 0.5 to 5 micrometers. Fungi are eukaryotes, which have hard cell walls. The size of fungi is variable; some may be tiny in size, while others form much larger structures. Protozoa are single-celled eukaryotic microorganisms. But protozoa do not have cell walls. Some protozoa are oval or spherical in shape, others are elongated. Cells can range in diameter from 1 μm to 2,000 μm, or 2 mm, and are difficult to observe without magnifying instruments (Michael 2016).

The most abundant microbes in the soil are generally bacteria, but due to their small size, they have a smaller biomass than actinomycetes. The difference is that the size of actinomycetes is 10 times that of bacteria, but their number decreased by 10 times, so their biomass is similar to that of bacteria. In addition, the fungal populations are smaller. These major microorganisms were disturbed differently under different temperature conditions. Bacteria, actinomycetes and protozoa are able to survive in colder conditions and tolerate more soil disturbances. Therefore, they dominate in tilled soils, while fungal and nematode populations are more prevalent in no-till soils (James 2010).

Nannipieri (2013) suggested that the central question posed by the link between microbial diversity and soil function is to understand the relationship between genetic diversity and community structure and between community structure and function. The structure and function of plant communities are determined by soil microorganisms. Because of their diversity, the rich and complex variety of soil microbes has many functions, such as their ability to be responsible for nitrogen fixation, nutrient cycling fixation that provides essential nutrients, and phytohormone production (Zabinski and Gannon 1997).

Throughout the ecosystem, bacteria are involved in many processes. For example, carbon cycling bacteria in both the atmosphere and soil have an important role. And in soils soil bacterial communities can be composed and diversified in relation to soil organic carbon (C) transformation processes (Jia *et al.,* 2020). The C and nitrogen (N) content of the soil also have an important role. In the soil N cycle, many bacteria (eubacteria and archaea) are involved in ammonification through a taxonomically narrow microbiota (Johannes and Erland 2011).

As one of the most diverse organisms on Earth, fungi are widely distributed in all terrestrial ecosystems (Zhang *et al.,* 2021). Fungi are diverse organisms that cover a wide range of forms from microscopic single-celled yeasts to large macrofungi. The functions of about 20 species of fungi have been described, and one of the main functions of fungi in soil is to act as major degraders (Rudgers *et al.,* 2014).

The process of decomposition of soil organic and mineral components requires the participation of fungi. Changes in the environment are closely related to the amount of soil microorganisms available (Jia *et al.,* 2020). For example, under drier and higher temperature conditions, not only can organic carbon utilization increase, but the microbial community can have a higher proportion of fungal biomass (Drenovsky *et al.,* 2003).

An increase in the number of fungi also leads to a concomitant increase in the rate of N mineralization. Higher N utilization may contribute to the positive correlation between plant diversity and productivity in N-limited soils, suggesting that plant-microbe interactions in soils are an integral component of the impact of plant diversity on ecosystem function (Donald *et al.,* 2003).

The diversity of microorganisms and the interactions among them are important in the various mass and energy exchanges that occur between the atmosphere, lithosphere, hydrosphere and biosphere. Microorganisms play an important role in environmental change and monitoring on a global scale. In addition, microorganisms have a key role in the treatment of pollution and bioprotection of microorganisms in all environments. Microorganisms are ubiquitous in soils and soil properties are greatly influenced (Jia *et al.,* 2020). For example, soil structure can be better maintained by soil microorganisms, the decomposition of various organic matter in the soil, the whole soil chemical cycle and the nutrient supply between soil and plants are also important

Many microorganisms (*e.g.,* mycorrhizal fungi or N-fixing symbiotic bacteria) play an important role in plant growth by improving mineral nutrition. Various structures have also been formed between plants and microorganisms, and secretions from plant roots react with microorganisms and vice versa. In continuous development, bacteria are adapting and are able to grow in different environments (Richard *et al.,* 2017).

2.3. Abiotic Effects on microbes

Soil type and soil nutrient factors are the most important determinants of soil microbial diversity (Michele *et al.,* 2011). In different natural environments, usually soil microbial communities are affected by the magnitude of fluctuations in temperature, rainfall, and other abiotic and biotic factors. Agricultural practices such as tillage can also have an impact on soil microbial communities.

Although bacteria are the most abundant group of soil microorganisms and play an important role in the material cycle and energy flow in the soil environment, fungi can be actively involved in the decomposition of organic matter. Fungi aid in decomposing proteins in dead litter, plant, and animal residues into nitrogen amino salts and ammonium salts that can be directly absorbed by vegetation. In addition fungi can help decompose cellulose, hemicellulose, lignin and other similar compounds, and is a key link in the carbon cycle. Actinomycetes can decompose most of the compounds that cannot be decomposed by fungi and bacteria, and also participate in the decomposition process of organic matter that is difficult to decompose.

2.3.1. Carbon

There is generally an energy source of C in the soil and soil microorganisms can be present in large quantities in the soil (James, 2010) when there is an optimal level of carbon. The entire amount of C storage has a direct role with soil microorganisms (Markus *et al.,* 2015). To obtain nutrients, plants depend on the growth of soil microorganisms such as bacteria and fungi that have metabolic mechanisms to depolymerize and mineralize organic forms of N, phosphorus (P), and sulphur (S; Richard *et al.,* 2017).

2.3.2. Temperature

The study of soil microbial community changes under global warming conditions is important for evaluating the global carbon cycle (Wang *et al.,* 2015). Temperature is an important factor affecting soil microbial activity, and an increase in soil temperature can result in significant changes in soil microbial biomass, activity and structure. Thus, the response of soil microbes to warming can be an important part of the ecosystem feedback process.

Linna (2012) argued that global climate change is generally expected to have an impact on soil conditions. Because changes in temperature will stimulate net primary production, the input of soil C will increase. The increased C input will affect soil microbial processes and plant growth. The addition of soil C and water will significantly affect microbial properties and stimulate plant growth. Therefore, studying the relationship between temperature changes and soil microorganisms can be more specific and helpful to explain plant growth.

In Yin's (1981) experiment, 309 test strains, including 203 bacteria, 27 actinomycetes, 47 fungi, and 32 other bacteria were used to investigate the effect of incubation temperature on the growth rate of different microorganisms and their culture characteristics. The final result was that the survival of all strains decreased if the temperature increased. However, the temperature sensitivity of bacteria and actinomycetes and other species differed.

3.0. MATERIALS AND METHODS

3.1. Quantitative Method

The study of the relationship between temperature and the content of microorganisms in the soil requires a research method through quantitative analysis. There are two variables in this study, one is the temperature change and the other is the change in the microbial content of the soil. Both of these variables can be expressed in the form of specific data so that the results can be better obtained by organizing and analyzing the data when studying the relationship. In this study, the quantitative analysis was used to obtain more accurate results than the qualitative analysis. Moreover, by analyzing the specific data, the results of the study can be more credible.

3.2. Study Site

Since 1994, Chongyang County has introduced *Phyllostachys praecon* from Zhejiang Lin'an City and Anji County, and now it has become the largest production base of *Phyllostachys praecon* in central China. The experimental data were collected in *Phyllostachys praecon* experimental forest created in 2000 (Luo *et al.,* 2016).

3.3. Data Choice

(Luo *et al.,* 2016) investigated the relationship between temperature and soil microbial content. The present data collection was carried out by first searching for published articles that have studied the relationship between the two. In this experiment, the numbers of soil bacteria, fungi and actinomycetes were measured, and the numbers of microorganisms were different at different temperatures, and the numbers of soil microorganisms were regularly distributed with the change of seasons. The temperature environment of Chongyang, a county with significant temperature differences between the four seasons, was able to demonstrate more clearly the trends in the number of soil microorganisms at different temperatures.

3.3.1. Data Collection

In January, April, July, and October 2014, Luo and her team sampled three standard plots of 5m×5m in a homogeneous *Phyllostachys praecon* stand. Soil profiles were then carefully excavated in appropriate plots around the standard plots, and soil samples were taken from each of the soil layers. Approximately 500 g of soil was taken separately in sterilized plastic bags and the sampling wasrepeated twice.

In 2014, Luo and others measured five major soil categories of microorganisms: bacteria, fungi, nitrogen-fixing bacteria, actinomycetes, and ammonia-forming bacteria. These five categories of soil microorganisms were counted in different ways. Bacteria: Beef paste peptone agar dilution mixed plate method with dilutions of 10-4, 10-5 and 10-6; fungi: Martin's medium dilution mixed plate method with dilutions of 10-2, 10-3 and 10-4; actinomycetes: Koch's 1 synthetic medium dilution mixed plate method with dilutions of 10-3, 10-4 and 10-5; N-fixing bacteria: modified; three replicates of each treatment were set up and incubated in a constant temperature incubator at 28°C for 36-72h for bacteria, 3-5d for fungi and 7-10d for actinomycetes, N-fixing bacteria were incubated for 5-7 d, and ammonia-forming bacteria for 3-5 d. The results were observed and counted separately.

The daily temperature of Chongyang County in 2014 in China was taken from the China Weather Forecast (CWF 2014). The temperatures for each day of the month were averaged to calculate the average temperature. The minimum and maximum temperatures for each day in the county were then obtained, and then the minimum and maximum temperatures for each month were averaged to make the data more concise. Then use the average of the minimum and maximum temperatures to get the overall average temperature for each month.

4.0. RESULTS

Examine differences in the populations of bacteria, fungi, actinomycetes, nitrogen-fixing bacteria and ammonifying bacteria in soils at different temperatures in 2014 (Luo *et al.,* 2016). In turn, it was determined whether or not there was a significant difference between them.

From Table 1, we can see that the overall number of the five microorganisms is the largest for bacteria, the second largest for fungi, and the smallest for actinomycetes.

Table 2. Number of microorganisms in different seasons(Luo *et al.,* 2016).

	Month (2014)			
Microorganism species	January ($x\ 10^4/g$)	April ($x\ 10^4/g$)	July ($x\ 10^4/g$)	October ($x\ 10^4/g$)
Soil bacteria	69.67	211.33	458.96	326.26
Soil fungi	0.15	2.66	4.38	0.84
Soil actinomycetes	1.63	50.67	7.68	5.38
Soil nitrogen-fixing bacteria	2.13	24.67	0.24	0.07
Soil ammoniating bacteria	1.01	24	7.02	3.6

The number of bacteria is much larger than that of fungi, but since the size of individual fungi is about ten times larger than that of bacteria and actinomycetes, the difference between the biomass of fungi and bacteria is not very large. The number of bacteria in each season is significantly different from the number of other microorganisms, and the number of bacteria is greater than that of other microorganisms.

For example, in July, the number of bacteria was 458.96 x 10^4/g, but the number of fungi was 4.38 x 10^4/g and the number of nitrogen-fixing bacteria was 0.24 x 10^4/g. Also, the number of the same categories of microorganism varied greatly from season to season, with the number of bacteria being 458.96 x 10^4/g in July but 69.67 x 10^4/g in January.

The average temperature of Chongyang County in 2014 can be seen in Table 2 and figure 1. The lowest temperatures were recorded in January as well as December, respectively. The highest temperatures are found in July, August and September.

Table 3. Temperature of Chongyang County in each month.

	Jan °C	Feb °C	Mar °C	Apr °C	May °C	Jun °C	Jul °C	Aug °C	Sep °C	Oct °C	Nov °C	Dec °C
Highest	13	9	19	24	27	30	33	32	29	26	18	13
Lowest	3	4	9	15	20	23	25	23	24	15	8	2
Average	8	6.5	14	20	24	27	29	28	27	21	13	8

The overall temperature change starts with a gradual warming in January, gradually reaching a maximum in July, followed by a gradual cooling to a minimum in December.

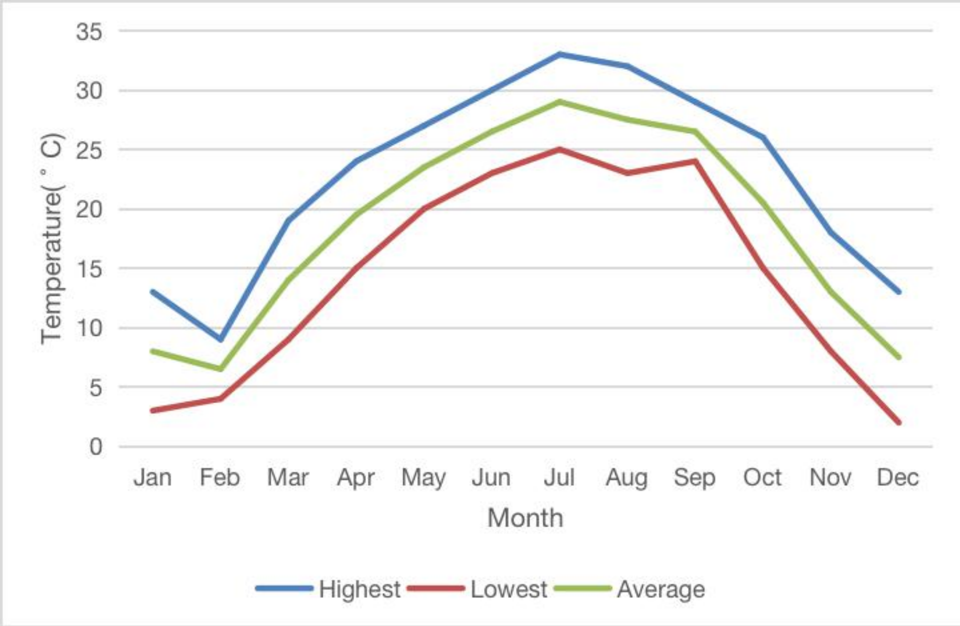

Figure 2. Minimum, maximum and average temperatures for each month.

Luo and her team (2016) chose to take the soil in January, April, July and October of 2014. This sampling method allows the data to be obtained at four different times of the year, making the structure of the experiment clearer.

In Figure 2, the change of pattern for the number of the five microorganisms was similar with a clear seasonal pattern. The lowest total number of microorganisms was found in January 2014, then it started to increase and reached the maximum total number of microorganisms in July 2014, then it started to decrease and the total number of microorganisms kept decreasing until October 2014.

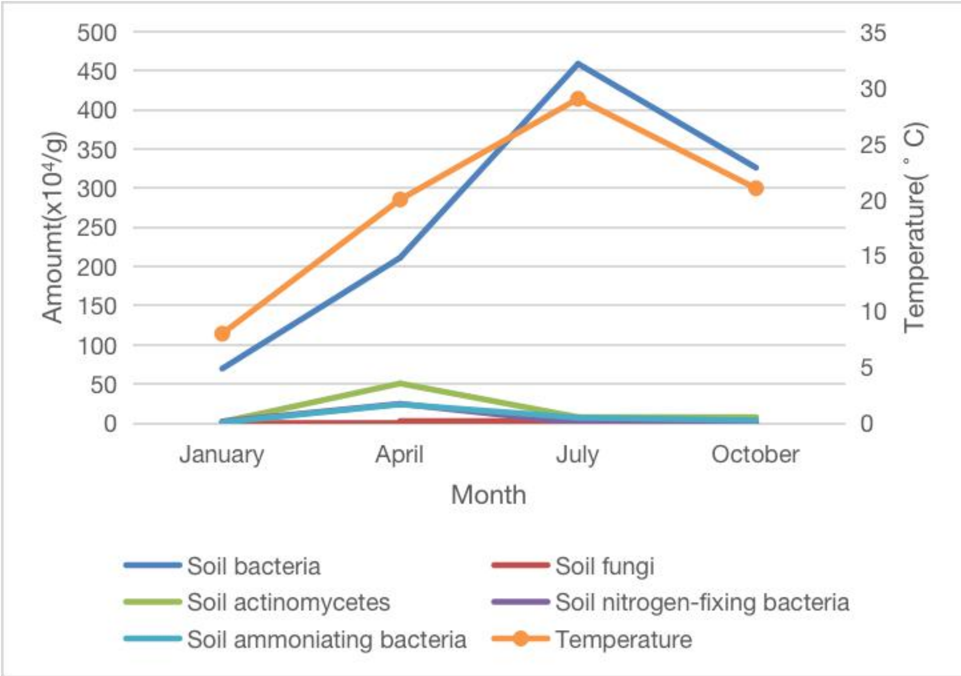

Figure 3. The relationship between different microbial contents and temperature.

5.0. DISCUSSION

After collecting the data and analyzing the results of the study, the soil studied at the study site had the largest number of bacteria, the second most amount of fungi, and the smallest number of actinomycetes among the three major categories of soil microorganisms. It was clear that bacteria had the highest number of the five microorganisms selected for the survey. The trend of bacteria basically determines the trend of total microorganisms since they make up the largest percentage of the total microorganisms.

The results of this study may be due to the gradual increase in temperature mainly from April onwards. As the weather temperature increased, the soil temperature also increased, a condition suitable for bacterial colonization. The same pattern of Biederbeck and Campbell (1973) proved the same for bacterial and actinomycete populations. The microbial population increased when the soil temperature was on the rise or remained at the same level for a full 28 days.

However, after July, the temperature gradually decreases. This temperature suppresses the activity of the microorganisms and the microorganisms die or become dormant, and most of them stop growing and reproducing, so the number of microorganisms decreases dramatically.

A gradual increase in the number of soil microorganisms as a component and producer of soil organic carbon can make a soil called 'good' soil (Christopher 2017). Plants can flourish due to the close association between bacteria and plants, and plant root secretions provide sufficient available nutrients for bacterial colonization. The environmental conditions for bacterial growth are adequately met and there is no competition between plants and microorganisms. In addition, microbial colonization accelerates the decomposition of organic matter on the surface, which further provides nutrients for bacteria (Kai *et al.,* 2016).

The relationship fits well with my hypothesis: as the temperature increases, the content of microorganisms in the soil increases; as the temperature decreases, the content of microorganisms decreases.

A limitation of this experiment lies in the quantitative analysis method used. For the experiment there are not many variables and the data collected is only a part of the overall study. The preliminary analysis based on these data is only able to show a relationship between the number of soil microorganisms and temperature in a specific area. The data are not broadly representative, so the final results cannot be used to make predictions over a wide range.

6.0. CONCLUSION

Data suitable for analysis were selected, and then the results were made clearer by analyzing the available data using tables and graphs. The main result of the study was the confirmation of a relationship between temperature and changes in soil microbial content. As the temperature increases, the number of soil microorganisms also increases. Therefore, this characteristic can be useful for plant growth, for example, by increasing the level of specific microorganisms during periods of elevated temperatures.

The method used is quantitative, which is limited by the fact that the results can only be obtained by analyzing the data and are not well correlated with real changes. In addition, the data collection in this study was not extensive, and the results are not applicable in a large scale.

Future studies should consider the possibility of using both quantitative and qualitative analyses so that they can analyze the problem more comprehensively. More reliable results can be obtained by analyzing more areas with more extreme temperature conditions.

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