American Journal of Plant Sciences, 2024, 15, 930-939
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

PLFA Analysis of Soil Microbial Community Structure in Different Forest Types

Jiayi Zhang[1,2], Songzhe Li[2], Xiangping Fu[3], Shunbao Lu[1,2], Yanjie Zhang[2*]

[1]Jiangxi Provincial Key Laboratory of Soil Erosion and Prevention, Nanchang, China
[2]College of Life Sciences, Key Laboratory of Poyang Lake Wetland and Watershed Research, Ministry of Education, Jiangxi Normal University, Nanchang, China
[3]College of Foreign Languages, Jiangxi Agricultural University, Nanchang, China
Email: *yanjiezhang0710@126.com

How to cite this paper: Zhang, J.Y., Li, S.Z., Fu, X.P., Lu, S.B. and Zhang, Y.J. (2024) PLFA Analysis of Soil Microbial Community Structure in Different Forest Types. *American Journal of Plant Sciences*, **15**, 930-939.
https://doi.org/10.4236/ajps.2024.1510059

Received: May 20, 2024
Accepted: October 25, 2024
Published: October 28, 2024

Abstract

Soil soluble organic matter is an important component in the study of carbon and nitrogen cycling in terrestrial ecosystems. Soil microorganisms, as soil decomposers, participate in soil biogeochemical processes and play an important role in maintaining the balance of soil ecosystems. As a typical subtropical regional unit, Queensland, Australia, is a relatively concentrated distribution area of forests in Australia. It is very sensitive to climate change and plays an important role in Australian climate and even global climate change. Its unique natural environment and ecosystem occupy a special position in the world. However, the knowledge of available carbon and nitrogen pool and microbial activity in forest soil is still very limited. *Pinus elliottii*, *Araucaria cunninghamii* and *Agathis australis* are the three most important forest types in southern Queensland, Australia. In our research, the function and structural diversity of soil microbial communities of these three forest types were studied using biochemical and molecular biological methods, and the effective carbon and nitrogen pools of soil of different forest types and related microbial processes were discussed, which has important theoretical guiding significance for further research on the structure and function of soil ecosystem. The number of PLFAs in the soil of *P. elliottii* was 45, the number of PLFAs in the soil of *Araucaria cunninghamii* and *Agathis australis* was 39 and 35, respectively. The number and content of PLFAs monomer in *P. elliottii* were higher than those in the other two kinds of forest soil.

Keywords

Forest Type, Soil, Microbial Community, PLFA

1. Introduction

Soil microbial community structure is closely related to the physical and chemical properties of soil, and the characteristics of soil microbial composition as an evaluation of soil quality change has attracted more and more attention [1] [2]. Phospholipids are a class of lipids containing phosphoric acid, which is the basic component of the cell membrane of living cells with diversity and biological specificity, and their composition and content are stable and hereditary in the same microorganism [3]-[5]. The phospholipids contained in soil microorganisms are mainly glycerol phospholipids, which are the main components of the phospholipid bilayer on the cell membrane of microorganisms. Phospholipids fatty acid (PLFA) is the fatty acid product obtained after phospholipids are extracted from methylated soil. It is gene-specific. Different microorganisms can form different PLFAs through different biochemical pathways, and some PLFAs always appear in the same group of microorganisms [3]. It is rarely seen in other types of microbes. When microorganisms die, fatty acids are rapidly metabolized, so the phospholipids in the soil are mostly in the form of components of living organisms [6]-[8]. The main factors affecting soil microorganisms are soil type, species composition, soil temperature and humidity, and soil management measures. Based on the above characteristics of phospholipid fatty acids, PLFA technology has been developed and widely used in the analysis of complex community structure of soil microorganisms, which is very suitable for the dynamic monitoring of microbial communities [9] and the response information of soil microbial communities to the environment [10]-[12], and the types of pure cultured microorganisms can be identified by the differences in the composition and content of fatty acids. This identification technique has become mature [13]-[15], and this method overcomes the shortcomings of traditional separation culture techniques.

In this paper, soil of different forest types in southeast Queensland, Australia was taken as the research object. PLFA technology was used to compare the changes of soil microbial community diversity of different forest types through the composition and content of fatty acids, so as to understand the effects of different forest types on soil microbial community diversity. It provides a basis for the scientific and rational use of soil to protect microbial diversity, the ecological functions of microorganisms in forest land and the sustainable development of forestry ecosystem.

2. Materials and Methods

2.1. Site

The test site was founded in 1921, located in the Sunshine Coast of southeast Queensland, Australia (25˚56'49"S, 153˚5'27"E) rolling hills and mountains, belonging to the subtropical monsoon climate. It is wet and cold in winter and dry and hot in summer. The average temperature is 12.5˚C in July (winter) and 31.2˚C in January (summer). The average annual precipitation is 741 - 2106 mm and the average precipitation is 1287 mm. The soil is a sandy loam from red soil to yellow soil, rich in minerals such as iron and aluminum [16], mainly derived from Mesozoic geological composition [17]. The soil is poor, loose in texture, strong in acidity, and low in cation exchange. However, the soil has good internal and external ventilation, abundant sunlight, low slope or flat land, and the slope direction is south slope, which is very suitable for plant growth. The main vegetation types are *P. elliottii*, *A. cunninghamii* and *A. australis*, *Eucalyptus globulus* and *Acacia confusa*. In this study, *P. elliottii*, *A. cunninghamii* and *A. australis* were mainly selected as the research objects. The quadrats were all pure forests, and the experimental area was 1.087, 0.308 and 0.428 hm^2, respectively. The canopy was small, the afforestation density was 140, 120 and 120 trees per hectare, and the tree height was more than 8 m. The understory vegetation is dominated by herbs such as *Herba verbenae*. The soil pH of the forest land of *P. elliottii*, *A. cunninghamii* and *A. australis* is 4.5, 6.0 and 6.2, respectively, and the soil of various plots is acidic. The sand content is above 95%, so the soil water retention performance is poor, and all are below 5%.

2.2. Sampling

The soil collection time was January 2009. Four 10 m × 20 m quadrates were set up in three different forest types of *P. elliottii*, *A. cunninghamii* and *A. australis*. In each quadrate, five pieces of soil (0 - 10 cm) were collected in an S-shape with a soil drill with a diameter of 7.5cm, then mixed, packed into a sealing bag and put into an incubator with ice cubes. The soil samples were screened 2 mm and stored in a refrigerator at 4˚C for the determination of soil carbon and nitrogen mineralization, microbial biomass, soil enzyme activity and soil soluble organic carbon and nitrogen. The other part is air-dried for the determination of soil grain size, soil total carbon, nitrogen and phosphorus and pH. Soil bulk density was measured by ring knife method.

2.3. Experimental Methods

The analysis method of phospholipid fatty acids was referred to scholar Wu Yuping [18], and the Total amount of phospholipid fatty acids (Total PLFA) was calculated by internal standard method. The effect between different treatments was tested for significance by variance. The data used in PCA analysis were the relative contents of PLFA.

2.4. Data Processing

SPSS 17.0 software was used to conduct one-way ANOVA on the data, LSD method was used to test the difference significance, Pearson correlation coefficient was used to evaluate the correlation between different factors, and Origin 7.5 and Sigmaplot 14 software were used for plotting. Canoco 4.5 software was used to perform redundancy analysis (RDA) on soil biological metabolism indexes and physical and chemical properties, and the significance level was $\alpha = 0.05$.

3. Results

3.1. PLFAs Contents of Soil Microorganisms in Different Forest Types

Phospholipid fatty acids are important components of the cell membrane of living microorganisms. Different groups of microorganisms can synthesize different PLFAs through different biochemical pathways. As can be seen from Table 1 and Figure 1, the number of PLFAs monomers in the soil of *P. elliottii* forest land is 45, and the number of PLFAs monomers in the forest land of *A. cunninghamii* and *A. australis* land is 39 and 35, respectively. The content of PLFAs in *P. elliottii* varied from 0.02 to 1.49 nmol·g^{-1}, accounting for 0.23% to 11.6% of the total PLFA content. The content of PLFAs in *A. cunninghamii* was 0.02 - 1.41 nmol·g^{-1}, accounting for 0.26% - 12.2% of the total PLFA content. The content of PLFA in *A. australis* was 0.03 - 0.82 nmol·g^{-1}, accounting for 0.43% - 11.8% of the total PLFA content. According to the above analysis, the number and content of PLFAs monomer in the forest soil of *P. elliottii* were higher than those of the other two tree species.

Table 1. The concentration PLFAs (nmol·g^{-1}) in different forest-type soil.

NO	PLFA	*P. elliottii* (nmol·g^{-1})	[b]%Total PLFA	*A. cunninghamii* (nmol·g^{-1})	%Total PLFA	*A. australis* (nmol·g^{-1})	%Total PLFA
1	14:00	0.18 ± 0.04	1.4	0.21 ± 0.05	1.82	0.15 ± 0.04	2.16
2	i14:0	0.03 ± 0.01	0.23	0.08 ± 0.01	0.69	0.05 ± 0.01	0.72
3	14:0 3OH	0.08 ± 0.03	0.62	ND[a]		ND	
4	15:00	0.16 ± 0.02	1.24	0.12 ± 0.02	1.04	0.05 ± 0.00	0.72
5	15:0 3OH	0.26 ± 0.15	2.02	ND		ND	
6	i15:0	0.74 ± 0.29	5.74	0.76 ± 0.13	6.57	0.42 ± 0.05	6.06
7	a15:0	0.22 ± 0.04	1.71	0.41 ± 0.06	3.54	0.35 ± 0.07	5.05
8	i15:1	0.17 ± 0.16	1.32	0.04 ± 0.02	0.35	ND	
9	15:1 w6c	0.05 ± 0.00	0.39	0.04 ± 0.01	0.35	0.06 ± 0.01	0.87
10	15:1 w8c	0.10 ± 0.03	0.78	ND		ND	
11	16:00	1.49 ± 0.23	11.6	1.41 ± 0.20	12.2	0.82 ± 0.06	11.8
12	16:0 2OH	0.05 ± 0.03	0.39	0.18 ± 0.05	1.56	0.09 ± 0.02	1.3
13	16:0 N alcohol	0.11 ± 0.02	0.85	0.04 ± 0.01	0.35	ND	
14	a16:0	0.02 ± 0.01	0.16	0.03 ± 0.01	0.26	0.03 ± 0.01	0.43
15	i16:0	1.01 ± 0.15	7.84	0.75 ± 0.15	6.48	0.32 ± 0.05	4.62
16	10Me16:0	1.09 ± 0.17	8.46	0.77 ± 0.12	6.66	0.38 ± 0.03	5.48
17	16:1 2OH	0.34 ± 0.05	2.64	0.02 ± 0.01	0.17	ND	
18	16:1 w5c	0.08 ± 0.01	0.62	0.18 ± 0.03	1.56	0.12 ± 0.01	1.73
19	16:1 w7c	0.53 ± 0.05	4.11	0.63 ± 0.06	5.45	0.32 ± 0.00	4.62
20	16:1 w9c	0.05 ± 0.02	0.39	0.06 ± 0.02	0.52	0.06 ± 0.03	0.87
21	i16:1	0.14 ± 0.01	1.09	0.14 ± 0.03	1.21	0.07 ± 0.03	1.01
22	17:00	0.07 ± 0.01	0.54	0.06 ± 0.01	0.52	0.04 ± 0.01	0.58

Continued

23	a17:0	0.19 ± 0.03	1.47	0.28 ± 0.04	2.42	0.17 ± 0.03	2.45
24	cy17:0	0.13 ± 0.03	1.01	0.25 ± 0.04	2.16	0.14 ± 0.01	2.02
25	10Me17:0	0.16 ± 0.03	1.24	0.14 ± 0.03	1.21	0.05 ± 0.01	0.72
26	i17:0	0.17 ± 0.03	1.32	0.26 ± 0.03	2.25	0.12 ± 0.02	1.73
27	17:1 w7c	0.17 ± 0.08	1.32	ND		ND	
28	17:1 w8c	0.11 ± 0.04	0.85	0.08 ± 0.01	0.69	0.05 ± 0.02	0.72
29	a17:1 w9c	ND		0.29 ± 0.10	2.51	ND	
30	a17:1	0.35 ± 0.13	2.72	ND		0.47 ± 0.27	6.78
31	18:00	0.48 ± 0.06	3.72	0.36 ± 0.05	3.11	0.24 ± 0.03	3.46
32	10Me18:0	0.23 ± 0.06	1.78	0.64 ± 0.06	5.53	0.27 ± 0.04	3.9
33	18:1 w5c	ND		0.26 ± 0.13	2.25	0.19 ± 0.07	2.74
34	18:1 w7c	0.40 ± 0.05	3.1	0.84 ± 0.19	7.26	0.59 ± 0.08	8.51
35	18:1 w9c	1.32 ± 0.16	10.2	0.99 ± 0.11	8.56	0.65 ± 0.07	9.38
36	11 Me 18:1 w7c	0.03 ± 0.01	0.23	0.13 ± 0.04	1.12	0.08 ± 0.01	1.15
37	18:2 w6, 9c	0.63 ± 0.08	4.89	0.30 ± 0.03	2.59	0.20 ± 0.05	2.89
38	18:3 w6c (6, 9, 12)	0.74 ± 0.59	5.74	0.06 ± 0.01	0.52	0.12 ± 0.07	1.73
39	cy19:0	1.09 ± 0.21	8.46	1.00 ± 0.19	8.64	0.64 ± 0.10	9.24
40	19:1 w6c	0.19 ± 0.05	1.47	ND		ND	
41	19:1 w11c	ND		0.11 ± 0.06	0.95	ND	
42	20:00	0.17 ± 0.04	1.32	0.08 ± 0.01	0.69	0.07 ± 0.01	1.01
43	20:4 w6, 9, 12, 15c	0.06 ± 0.02	0.47	ND		0.03 ± 0.02	0.43
	Total content	12.89 ± 1.94	100	11.57 ± 1.68	100	6.93 ± 0.84	100
Number of individual PLFA		45		39		35	

Data: Mean ± S.E., [a]ND: not detected, [b]%total PLFAs: percentage of this over total PLFAs.

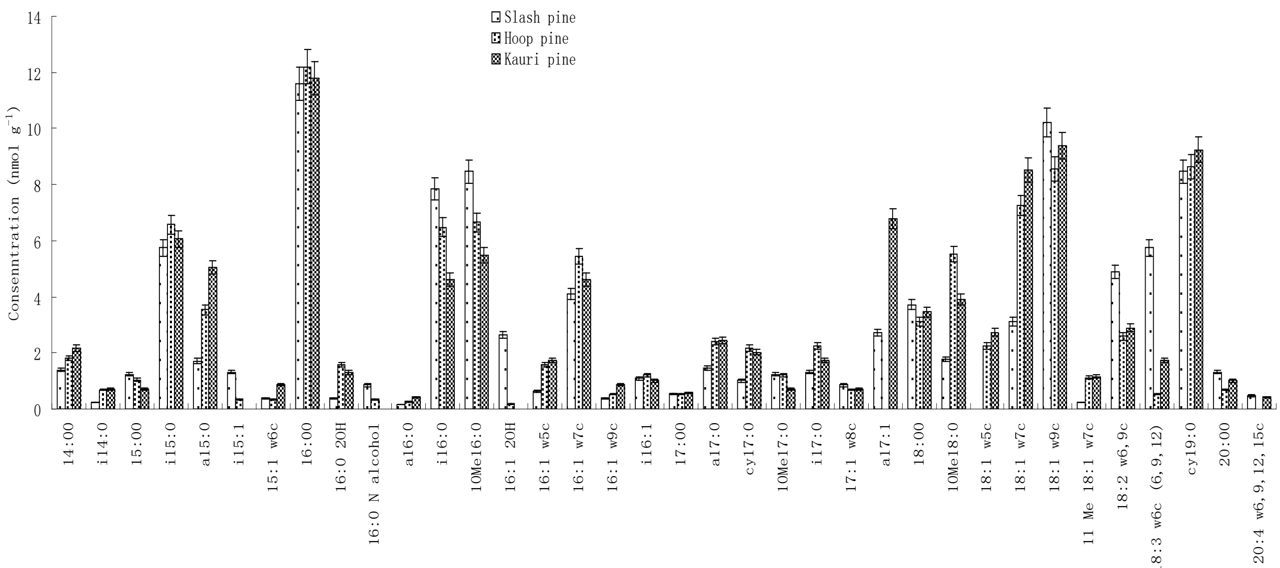

Figure 1. The concentration of individual PLFAs detected in different forest soils. Note: Only those PLFAs detected in different soils are include.

3.2. Analysis of Soil Microbial Community Diversity in Different Forest Types

In the study of soil microbial community structure, PLFAs analysis has received extensive attention. A principal component analysis was performed for all PLFAs (the number of PLFAs individuals measured in soil) in three different soil samples (**Figure 2(a)** and **Figure 2(b)**). However, in PCA analysis of all or a total PLFAs content, there must be no missing data in the data matrix. For the missing data in this study, we replaced the undetected PLFAs with 0. PCA results of PLFAs in soil of different forest types showed that, the phospholipid fatty acid profiles characterized by all PLFAs and common PLFAs were very similar (**Figure 2(a)** and **Figure 2(b)**), and the results reflected similar regular changes in PLFAs profiles with different forest types, that is, the value of the first principal component of *P. elliottii* was the lowest, that of *A. cunninghamii* was the middle, and that of *A. australis* was the highest. The degree of variation reflected by the first principal component of PLFA spectra of all PLFAs and common PLFAs was 38.99% and 50.95%, respectively.

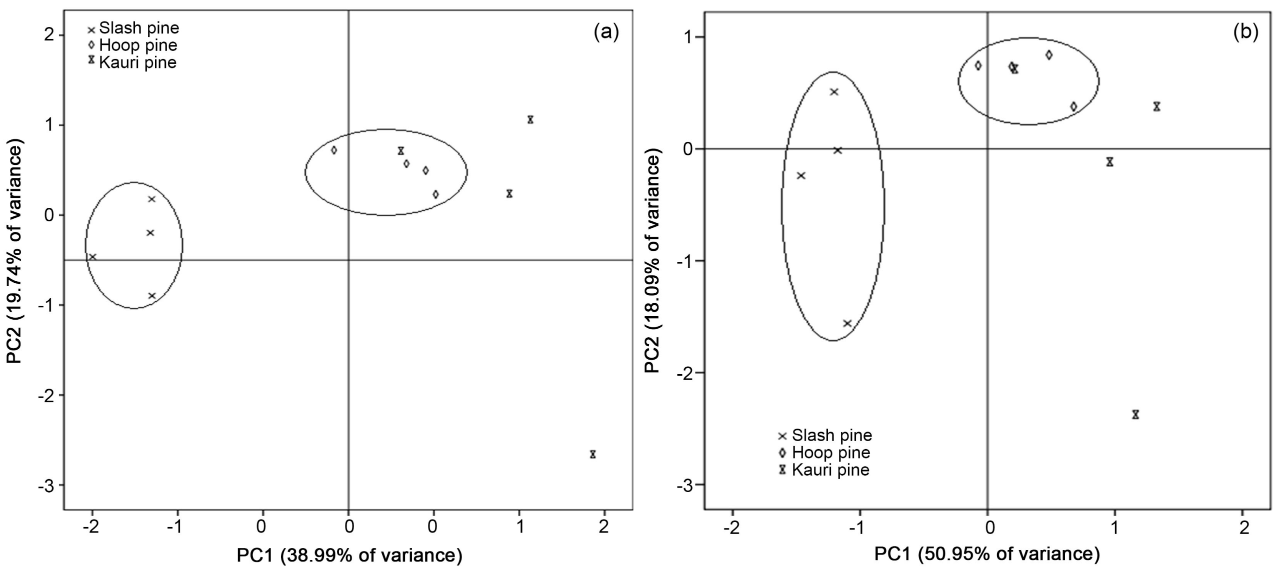

Figure 2. Principal component analysis (PCA) of PLFAs profiles extracted from different soil from. (a)with all the individual PLFAs or (b)the common PLFAs.

3.3. Relative Content of Biomarkers PLFAs in Soils of Different Forest Types

Total phospholipid fatty acid (total PLFA) was used to characterize soil microbial biomass. Here fatty acids 15:0, 17:0, i15:0, i16:0, i17:0, a15:0, A17:0, 16:1*ω*7c, 18:1*ω*7c, cy17:0 and cy19:0 are used to indicate bacterial PLFAs; 18:2 and 18:3 were used to indicate PLFAs; Monounsaturated fatty acids and cyclopropane fatty acids 16:l*ω*5c, 16:1*ω*7c, 16:1*ω*9c, 17:1*ω*8c, 18:1*ω*5c, 18:1*ω*7c, 18:1*ω*9c, cy17:0, cy19:0 were used to indicate gram-negative bacteria PLFAs. Branched-chain and saturated fatty acids i14:0, i15:0, i16:0, i17:0, a15:0, al7:0 were used to indicate gram-positive bacteria PLFAs. The following fatty acid ratios were also used in this study, the ratio of cyclopropane fatty acid to its precursor fatty acid[(cy: pre), (cy17:0 + cy19:0): (16:1ω7 + 18:1ω7)]; The ratio of isomeric PLFAs to anti-isomeric PLFAs[(I: a), (i15:0 + i17:0): (a15:0 + a17:0)], the ratio of saturated fatty acids to monounsaturated fatty acids [(sat: mono), (14:0 + 15:0 + 16:0 + 17:0 + 18:0 + 20:0): (now omega 9 c + now omega 7 c, c + + now omega 5 now omega 8 c + and omega 9 c + and omega 7 c, c + + and omega 5 19:1 11 c + omega 9 c) all the sons of omega]. Analysis of the relative content of biomarker PLFAs in different forest soils showed that the content of soil in different forest types was not significant (**Table 2**). The content of bacterial PLFAs in the three forest soils was, *A. cunninghamii* > *A. australis* > *P. elliottii*, while the content of fungal PLFAs was *P. elliottii* > *A. australis* > *A. cunninghamii*. The content of gram-negative PLFAs was the highest in *A. australis* soil, followed by *A. cunninghamii* soil, and the lowest in *P. elliottii* soil. There was little difference in the content of gram-positive bacteria in the soil of *P. elliottii* and *A. cunninghamii*, but it was lower in the soil of *A. australis*. Three groups of proportional fatty acids (the ratio of propane fatty acid to its precursor PLFAs, the ratio of isomeric PLFAs to trans-isomeric PLFAs, and the ratio of saturated fatty acid monounsaturated fatty acids) are commonly used as indicators of environmental stress. The ratio of propane fatty acids to its precursor PLFAs and the ratio of isomeric PLFAs to trans-isomeric PLFAs were *A. australis* > *A. cunninghamii* > *P. elliottii*, while the ratio of saturated fatty acids to monounsaturated fatty acids had little change.

Table 2. The relative contents of biomarker PLFAs in three forest soil.

biomarker PLFAs	*P. elliottii* (%)	*A. cunninghamii* (%)	*A. australis* (%)
bacteria	22.84	24.01	22.91
fungi	6.625	1.611	2.322
Gram-negative bacteria	17.96	19.22	20.02
Gram-positive bacteria	11.44	11.4	10.35
Cy:pre	10.44	12.18	12.26
I:a	6.375	7.668	7.688
Sat:mono	24.44	24.15	24.27

Cy:pre, the ratio of cyclopropane PLFAs to their precursors; I:a, the ratio of isomeric to anti-isomeric PLFAs; Sat:mono, ratio of saturated fatty acids to monounsaturated fatty acids.

4. Discussion

In the forest ecosystem, a high-quality soil, abundant microbial species, should have good biological activity and stable microbial population composition. These microbial species directly participate in the inorganic and humic processes of soil organic matter. Decompose organic matter, release nutrients, and promote plant growth. and soil microorganisms are a huge driving force for nutrient sources and sinks in the soil ecosystem, so soil microorganisms play an important role in litter decomposition and nutrient cycling [19] [20]. When extracting PLFAs from soil, they are likely to come from soil humus, which is a long chain fatty acid containing ester linkages, not from microbial cells. This led to the extraction of non-living PLFAs [21]. The PLFAs extraction method adopted in this study can extract PLFAs from soil to the maximum extent. However, the study results showed that the number of PLFAs detected in forest soil of *A. cunninghamii* was lower than that of the other two tree species (**Table 1**), possibly because the decomposition products of forest litter and the low soil water content of *A. cunninghamii* limited the growth and propagation of soil microorganisms. The soil organic matter content of *P. elliottii* is higher than that of the other two forest types, and the color of the soil extract is yellow, indicating that it contains some humus substances. In addition, soil preservation may also have certain differences in the number of PLFAs monomers. Since the soil is directly stored in cold storage after collection, low temperature is likely to have certain effects on cells, such as the rapid degradation of cell membranes after cell death, and the phospholipids contained in them are also rapidly metabolized. However, this situation does not occur under extreme environmental conditions. For example, there are more suitable conditions such as water content and temperature [22]. At −70˚C, even if cells die, their tissue components are preserved to a large extent, and the phospholipid content in this experiment is reduced at 4˚C, which is speculated to be due to degradation, but the objective reason remains to be explored. The difference between this result and the results of this study may be related to the preservation time and method. Therefore, in order to obtain comparable PLFAs results in the same set of experiments, it is recommended to analyze the same soil in as short a time as possible to reduce the error caused by preservation.

5. Conclusion

The number of PLFAs in the soil of *P. elliottii* was 45, the number of PLFAs in the soil of *A. cunninghamii* and *A. australis* forest was 39 and 35, respectively. The content of PLFAs in the forest land of *P. elliottii* varied from 0.02 to 1.49 $nmol \cdot g^{-1}$, accounting for 0.23% to 11.6% of the total PLFA content. The content of PLFAs in *A. cunninghamii* forest was 0.02 - 1.41 $nmol \cdot g^{-1}$, accounting for 0.26% - 12.2% of the total PLFA content. The content of PLFA in *A. australis* forest was 0.03 - 0.82 $nmol \cdot g^{-1}$, accounting for 0.43% - 11.8% of the total PLFA content. According to the above analysis, the number and content of PLFAs monomer in the forest soil of *P. elliottii* were higher than those of the other two tree species. The analysis of the relative content of biomarkers PLFAs in different forest soils showed that the content of different forest types was not obvious. The content of bacterial PLFAs in the three forest soils was *A. cunninghamii* > *A. australis* > *P. elliottii*, while the content of fungal PLFAs was *P. elliottii* >*A. australis* > *A. cunninghamii*. The content of gram-negative PLFAs was the highest in *A. australis* soil, followed by that in *A. cunninghamii* soil, and the lowest in *P. elliottii* soil. There was little difference in the content of gram-positive bacteria in the soil of *P. elliottii* and fir, but it was lower in the soil of *A. australis*.

Funding

The work was funded by National Natural Science Foundation of China (32260297), Natural Science Foundation of Jiangxi Province (20224ACB205003). Jiangxi Provincial Key Laboratory of Soil Erosion and Prevention (2021SKTR05) and Key Laboratory of Poyang Lake Wetland and Watershed Research of Jiangxi Normal University (PK2021002). The authors take this opportunity to thank all for the support extended for the research.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G. and Renella, G. (2003) Microbial Diversity and Soil Functions. *European Journal of Soil Science*, **54**, 655-670. https://doi.org/10.1046/j.1351-0754.2003.0556.x

[2] Tunlid, A., Baird, B.H., Trexler, M.B., Olsson, S., Findlay, R.H., Odham, G., *et al.* (1985) Determination of Phospholipid Ester-Linked Fatty Acids and Poly B-Hydroxybutyrate for the Estimation of Bacterial Biomass and Activity in the Rhizosphere of the Rape Plant *Brassica napus* (L.). *Canadian Journal of Microbiology*, **31**, 1113-1119. https://doi.org/10.1139/m85-210

[3] Haack, S.K., Garchow, H., Odelson, D.A., Forney, L.J. and Klug, M.J. (1994) Accuracy, Reproducibility, and Interpretation of Fatty Acid Methyl Ester Profiles of Model Bacterial Communities. *Applied and Environmental Microbiology*, **60**, 2483-2493. https://doi.org/10.1128/aem.60.7.2483-2493.1994

[4] Haldeman, D.L., Amy, P.S., Ringelberg, D., White, D.C., Garen, R.E. and Ghiorse, W.C. (1995) Microbial Growth and Resuscitation Alter Community Structure after Perturbation. *FEMS Microbiology Ecology*, **17**, 27-38. https://doi.org/10.1111/j.1574-6941.1995.tb00124.x

[5] Lundquist, E.J., Scow, K.M., Jackson, L.E., Uesugi, S.L. and Johnson, C.R. (1999) Rapid Response of Soil Microbial Communities from Conventional, Low Input, and Organic Farming Systems to a Wet/Dry Cycle. *Soil Biology and Biochemistry*, **31**, 1661-1675. https://doi.org/10.1016/s0038-0717(99)00080-2

[6] White, D.C., Davis, W.M., Nickels, J.S., King, J.D. and Bobbie, R.J. (1979) Determination of the Sedimentary Microbial Biomass by Extractible Lipid Phosphate. *Oecologia*, **40**, 51-62. https://doi.org/10.1007/bf00388810

[7] Frostegård, Å., Tunlid, A. and Bååth, E. (1993) Phospholipid Fatty Acid Composition, Biomass, and Activity of Microbial Communities from Two Soil Types Experimentally Exposed to Different Heavy Metals. *Applied and Environmental Microbiology*, **59**, 3605-3617. https://doi.org/10.1128/aem.59.11.3605-3617.1993

[8] Kasurinen, A., Keinänen, M.M., Kaipainen, S., Nilsson, L., Vapaavuori, E., Kontro, M.H., *et al.* (2005) Below-Ground Responses of Silver Birch Trees Exposed to Elevated CO_2 and O_3 Levels during Three Growing Seasons. *Global Change Biology*, **11**, 1167-1179. https://doi.org/10.1111/j.1365-2486.2005.00970.x

[9] Balkwill, D.L., Leach, F.R., Wilson, J.T., McNabb, J.F. and White, D.C. (1988) Equivalence of Microbial Biomass Measures Based on Membrane Lipid and Cell Wall Components, Adenosine Triphosphate, and Direct Counts in Subsurface Aquifer Sediments. *Microbial Ecology*, **16**, 73-84. https://doi.org/10.1007/bf02097406

[10] Zelles, L. and Bai, Q.Y. (1993) Fractionation of Fatty Acids Derived from Soil Lipids by Solid Phase Extraction and Their Quantitative Analysis by Gc-Ms. *Soil Biology and Biochemistry*, **25**, 495-507. https://doi.org/10.1016/0038-0717(93)90075-m

[11] von Rein, I., Gessler, A., Premke, K., Keitel, C., Ulrich, A. and Kayler, Z.E. (2016) Forest Understory Plant and Soil Microbial Response to an Experimentally Induced Drought and Heat-Pulse Event: The Importance of Maintaining the Continuum. *Global Change Biology*, **22**, 2861-2874. https://doi.org/10.1111/gcb.13270

[12] Lechevalier, M.P. and Moss, C.W. (1977) Lipids in Bacterial Taxonomy—A Taxonomist's View. *CRC Critical Reviews in Microbiology*, **5**, 109-210. https://doi.org/10.3109/10408417709102311

[13] Hackl, E., Pfeffer, M., Donat, C., Bachmann, G. and Zechmeisterboltenstern, S. (2005) Composition of the Microbial Communities in the Mineral Soil under Different Types of Natural Forest. *Soil Biology and Biochemistry*, **37**, 661-671. https://doi.org/10.1016/j.soilbio.2004.08.023

[14] Findlay, R.H. (1996) The Use of Phospholipid Fatty Acids to Determine Microbial Community Structure. In: Akkermans, A.D., van Elsas, J.D. and de Bruijn, F.J., Eds., *Molecular Microbial Ecology Manual*, Springer, 77-93. https://doi.org/10.1007/978-94-009-0215-2_7

[15] Grayston, S.J. and Prescott, C.E. (2005) Microbial Communities in Forest Floors under Four Tree Species in Coastal British Columbia. *Soil Biology and Biochemistry*, **37**, 1157-1167. https://doi.org/10.1016/j.soilbio.2004.11.014

[16] Isbell, R.F. (1996) The Australian Soil Classification. CSIRO Publishing.

[17] Simpson, J. and Osborne, D. (2006) Performance of Seven Hardwood Species Underplanted to *Pinus elliottii* in South-East Queensland. *Forest Ecology and Management*, **233**, 303-308. https://doi.org/10.1016/j.foreco.2006.05.021

[18] Wu, Y.P. (2009) Studies on Soil Microbial Community Structure Based on Phospholipid Fatty Acid (PLFA) Analysis. Zhejiang University.

[19] Kemmitt, S.J., Lanyon, C.V., Waite, I.S., Wen, Q., Addiscott, T.M., Bird, N.R.A., *et al.* (2008) Mineralization of Native Soil Organic Matter Is Not Regulated by the Size, Activity or Composition of the Soil Microbial Biomass—A New Perspective. *Soil Biology and Biochemistry*, **40**, 61-73. https://doi.org/10.1016/j.soilbio.2007.06.021

[20] Polymenakou, P.N., Bertilsson, S., Tselepides, A. and Stephanou, E.G. (2005) Links between Geographic Location, Environmental Factors, and Microbial Community Composition in Sediments of the Eastern Mediterranean Sea. *Microbial Ecology*, **49**, 367-378. https://doi.org/10.1007/s00248-004-0274-5

[21] Schnitzer, M. and Neyroud, J.A. (1975) Alkanes and Fatty Acids in Humic Substances. *Fuel*, **54**, 17-19. https://doi.org/10.1016/0016-2361(75)90023-x

[22] Lu, S.B. (2011) Soil Labile Organic Carbon and Nitrogen Pools and Associated Microbial Process under Three Forest Types in Australia. Jiangxi Agriculture University.