forests

MDPI

Article

Effects of Habitat Differences on Microbial Communities during Litter Decomposing in a Subtropical Forest

Hongrong Guo, Fuzhong Wu, Xiaoyue Zhang, Wentao Wei, Ling Zhu, Ruobing Wu and Dingyi Wang *

Key Laboratory for Humid Subtropical Eco-Geographical Processes of the Ministry of Education, School of Geographical Sciences, Fujian Normal University, Fuzhou 350007, China; qsx20201062@student.fjnu.edu.cn (H.G.); wufzchina@fjnu.edu.cn (F.W.); qbx20210127@student.fjnu.edu.cn (X.Z.); qsx20201041@student.fjnu.edu.cn (W.W.); qsx20201049@student.fjnu.edu.cn (L.Z.); qsx20201025@student.fjnu.edu.cn (R.W.)
* Correspondence: albertwdy@fjnu.edu.cn

Abstract: The differences between aquatic and terrestrial habitats could change microbial community composition and regulate litter decomposition in a subtropical forest, but the linkage remains uncertain. Using microbial phospholipid fatty acids (PLFAs), the litter decomposition associated with microbial organisms was monitored to characterize the differences of microbial communities in the forest floor, headwater stream, and intermittent stream. Habitat type did not significantly affect the concentrations of total PLFA. However, microbial community composition (fungi, G+ bacteria, and eukaryote) was significantly affected by the microenvironment among habitats. Compared with which in headwater stream, more individual PLFAs were identified in the natural forest floor and the intermittent stream during the whole decomposition period. The differences in individual PLFA concentrations were reflected in the forest floor and aquatic system in the early stage of litter decomposition, but they mainly reflected in the headwater stream and the intermittent stream in the later stage of litter decomposition. We linked the relationships between microbial community and litter decomposition and found that communities of decomposers drive differences in litter decomposition rate among habitats. Intriguingly, the microbial community showed the greatest correlation with the decomposition rate of litter in streams. These findings could contribute to the understanding of habitats difference on the microbial community during litter decomposition.

Keywords: leaf litter decomposition; habitat types; microbial community; phospholipid fatty acids (PLFAs); individual PLFA

check for updates

Citation: Guo, H.; Wu, F.; Zhang, X.; Wei, W.; Zhu, L.; Wu, R.; Wang, D. Effects of Habitat Differences on Microbial Communities during Litter Decomposing in a Subtropical Forest. *Forests* **2022**, *13*, 919. https://doi.org/10.3390/f13060919

Academic Editor: Bartosz Adamczyk

Received: 10 April 2022
Accepted: 8 June 2022
Published: 13 June 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Litter decomposition, a crucial component of carbon and nutrient cycles, plays an indispensable role in regulating ecosystem structure and function in forests [1]. The litter produced by plants in terrestrial ecosystems most reaches the forest floor. Meanwhile, there are abundant headwater streams and intermittent streams in forest ecosystems. These aquatic ecosystems can receive substantial inputs of terrestrially-derived leaf litter. As a result, streams reconnect terrestrial and aquatic ecosystems by flows of organic matter that represent a great contribution to the global carbon cycle [2]. Some microbial decomposer communities, including fungi and bacteria, drives leaf litter decomposition which controls the flow of energy and nutrients in forest ecosystems [3,4]. Due to the different sensitivity of bacteria and fungi, they respond differently to relatively rapid shifts in microenvironmental conditions, such as temperature, precipitation, dissolved oxygen, or pH which affects their relative contribution to leaf decomposition [5,6]. After entering the ecosystems, habitats differ in properties such as nutrient availability, major environmental conditions, processes, and dynamics, which altogether influence the microbial abundance and community composition, and ultimately contribute to the decomposition process of litter [7,8]. Unfortunately, studies on microbial communities in litter decomposition have often been focused only on individual terrestrial or aquatic ecosystems, and only

a handful of studies have dealt with the differences between them. This hinders our understanding of the impact of habitat differences on microbial community changes during litter decomposition.

The microenvironment in which microbial decomposers survive varies greatly between terrestrial and aquatic systems, due to the contrasting spatial and temporal scales at which litter is decomposed in different habitats [9]. These differences can control microbial decomposition by regulating microbial decomposers. Specifically, on the forest floor, the litter is in direct contact with the soil surface. At this time, the surface temperature and rainfall are important environmental factors to regulate the activities of microbial decomposers in terrestrial ecosystems [10]. Meanwhile, the microtopography, exposition, animal activities, or plant presence will also lead to the variations of temperature and humidity at regional scales of the forest floor [11] and then affect the activities of microbial decomposers. Compared with terrestrial ecosystems, the headwater stream is typified by relatively stable temperature, sufficient water source, low oxygen, and strong scour, which regulate microbial community composition [12,13]. When entering a stream, leaf litter will be promptly colonized by a diverse array of microbial decomposers, particularly aquatic fungi [14]. The hydrological regimes of intermittent streams are characterized by alternating flowing, nonflowing, and dry phases [13,15]. The microbial decomposer in litter decomposition in intermittence streams could be similar to headwater streams during flowing phases while it may be similar to the forest floor during dry phases. When it comes to the dry phases, the litter will be exposed to solar radiation, and its temperature and humidity will change dynamically [16,17]. Once the dry phase finishes, gradual rewetting promotes the onset of microbial decomposition [18]. As a result, microbial decomposition processes and rates dramatically change among these different habitat types but need further and unified understanding.

The phospholipid fatty acids (PLFAs) analysis method has been used for more than 42 years and is still popular as a means to characterize microbial communities in a diverse range of environmental matrices [19]. It is considered that degradation of PLFA biomarkers proceeds rapidly after cell death, providing robust information on the living microbial biomass and community structure [20]. Thus, the approach is advantageous there in that it can identify living microbial biomass and is more sensitive in detecting shifts in the microbial community compared to DNA-/RNA-based methods [21]. In addition, PLFA could provide more information on such a variety of microbial characteristics (both functional and structural) in a single analysis than other methods [19].

South China is rich in hydrothermal resources and breeds the world's largest humid subtropical mountain forest, which is typified by fast material circulation, rich biodiversity, and high productivity [22]. There are dense headwater streams hidden between mountains and gullies, and abundant intermittent streams on the forest surface in the rainy season [23]. Under the influence of water collection, a large number of forest litter gather in intermittent streams and headwater streams [24]. Microorganism plays a vital role in the biological decomposition of leaf litter in aquatic and terrestrial ecosystems. Due to its sensitivity to environmental conditions, microbial biomass and community composition may be different, and they ultimately influence the decomposition process of litter. Therefore, we hypothesized that the unique environmental conditions in different habitats will change the microbial community structure and influence the decomposition process of litter. To address this hypothesis, we collected leaf litter of the typical tree species *Castanopsis carlesii* (Hemsl.) Hayata. and use the litter bag decomposition method in the natural forest floor, headwater stream, and intermittent stream of the subtropical area. The intention of this study is not only to focus on the changes of microbial community in the process of litter decomposition in a single habitat but to focus on the main differences between habitats and its influence. This may help to improve our understanding of the abiotic drivers (microbial decomposers) on litter decomposition in forest ecosystems.

2. Materials and Methods

2.1. Study Site

This study was conducted at the Sanming Research Station of Forest Ecosystem, Fujian Province, China (26°19′ N, 117°36′ E). This area is dominated by low mountains with an average elevation of approximately 300 m and slopes of 25–45°. The soil is red soil with a thickness of more than 1 m. The climate is a maritime subtropical monsoon climate, with a mean annual temperature and precipitation of 19.3 °C and 1610 mm. Approximately 80% of rainfall occurs between March and August. The natural vegetation is a subtropical evergreen broadleaved forest. The nature *Castanopsis carlesii* is the dominant species, with *Schima superba* Gardn. et Champ., *Castanopsis fissa* (Champion ex Bentham) Rehder et E. H. Wilson, *Litsea elongate* (Wall. ex Nees) Benth. et Hook. f., and *Neolitsea aurata* (Hay.) Koidz. in the forest canopy and *Ormosia xylocarpa* Chun ex L. Chen, *Itea chinensis* Hook. et Arn., and *Ilex pubilimba* Merr. et Chun in the shrub layer.

2.2. Foliar Litter Decomposition Experiment

In January 2021, the fresh senescent foliar litter from *C. carlesii* was collected from the forest floor at the sample sites. The initial dry mass was obtained by measuring litter samples that were oven-drying (65 °C, 48 h) after being air-dried for more than 2 weeks at room temperature. Samples of air-dried foliar litter were placed inside nylon mesh bags (20 cm × 20 cm; 1.0 mm mesh size nylon mesh of bag; 10.00 g per bag) [25]. On 28 March 2021, litterbags were placed in three different habitats, including forest floor, headwater stream, and intermittent stream, respectively.

Three natural *C. carlesii* forest plots were selected for setting a 3 m × 3 m homogeneous quadrat in each site as three sample repeated plots. Before the formal experiments, we investigated the annual accumulation of litter per unit area one year and loaded required weight according to the area of the litter bag. Litter bags were placed on the forest floor after removing plants and litter from the soil surface of the quadrat. An interval of at least 2 cm was placed between each litterbag to avoid mutual disturbance upon collection. At a distance of about 200 m from the natural forest sample plot, three representative intermittent streams with basically the same environmental conditions, about 200 m apart and concentrated natural litter, were randomly selected as three repeated sample plots to place litter bags. The withered leaf bag is fixed to the root of the main branch of the shrub in the sample belt through the safety rope to prevent scouring and falling off. A stream about 100 m away from the natural forest sample was selected. Three points are randomly selected from the upstream to the downstream as repeated sample plots. Each sample plot is 1–2 km away from each other. The safety rope tied with litter bags is placed in the water body in the center of the stream, which is similar to the distribution of litter under natural conditions and can fluctuate with the flow of water. The safety rope is fixed at the main root of tall trees in the sample zone. As a result, a total of 98 litterbags (3 samples × 12 sampling dates (a whole year) × 3 replicates) were prepared in each habitat. All litter bags in terrestrial and aquatic ecosystems were carefully placed for avoiding overlap among samples.

After placing in the plots, three litter bags of each site were sampled and immediately brought back to the laboratory for refrigeration at the end of each month from April. However, due to the fast decomposition rate of the headwater stream and the intermittent stream, the litter is decomposed within 4 months. At the same time, we also chose to analyze the litter decomposition on the forest surface for four months. The specific sampling date is the 28th, 65th, 92nd, and 119th days after decomposition. Foreign materials, such as roots, soil debris, lichen, and bryophyte, were carefully removed from the litterbags. We freeze-dried (Wizard 2.0 hot plate vacuum freeze dryer VirTisUSA) all the collected samples until constant weight (about 3–4 days), which is used for the determination of residue rate. After that, three bags of litter in the same sample plots were mixed and ground and placed at −80 °C for the determination of PLFA (n = 3).

2.3. Monitoring Environmental Conditions

At the beginning of the experiment, rainfall buckets and thermohygrometers were set up in the sample plot to record the rainfall and atmospheric temperature every 1 h and 0.5 h respectively. The headwater stream water temperature was also monitored. Water samples from the headwater stream and intermittent stream were collected to determine the pH value. At the same time, according to rainfall data and water samples collected in intermittent streams in the same period, we found that the hydrological regimes of intermittent stream showed seven times alternating flowing during litter decomposition (during 31 March 2021–3 April 2021, 8–11 April 2021, 14 April 2021–7 July 2021, and 23–24 July 2021, there was water flow for 93 days in total; during 4–7 April 2021, 12–13 April 2021, and 8–23 July 2021, there was no water flow for 21 days in total).

2.4. PLFA Analysis

Total lipids were extracted from 1.0 g freeze-dried litter soaked in a mixture of chloroform: methanol: phosphate buffer (1:2:0.8, $v/v/v$) according to the method of Zelles and Otaki [26,27]. Phospholipids were isolated on silica columns and hydrolyzed and methylated using a methanolic KOH solution. Fatty acid methyl esters (FAME) were separated into saturated, polyunsaturated and monounsaturated fatty acids using amino propyl-modified and silver-impregnated SPE columns. The PLFA module of the American MIDI Sherlock microbial identification system platform is used for sample analysis, with hydrogen as the carrier gas, Agilent 6890N meteorological chromatograph and FID detector as the hardware platform. The chromatographic column is Agilent 19091B-102 (25 m $\times$ 200 μm $\times$ 0.33 μm). The injection volume of each sample is 1 μL. According to the MIDI platform specification, all tests are calibrated with standards. PLFAs were identified and analyzed based on the MIDI map recognition software Sherlock system 6.2.

2.5. Data Analysis

We examined how habitats impacted the concentrations of microbial PLFAs and the ratios of biomarkers by using repeated measures MANOVA with habitat type and decomposition time as factors. We used one-way ANOVA in the case of normal distribution of data and nonparametric test in the case of non-normal distribution of data to compare the differences of microbial PLFAs concentrations among habitats in the same decomposition period. Microbial community structure was characterized by performing principal components analysis (PCA) on the $\log_{10}$-transformed mole of various microbial groups PLFA biomarkers. Subsequently, we used linear regression to analyze the influence of habitat type on the first two principal component scores obtained after PCA of various microbial groups PLFA biomarkers. Meanwhile, we selected individual PLFA that simultaneously acted on litter decomposition during the whole decomposition period in three habitats. We selected individual PLFA that acts on three habitats in the same decomposition period to compare the number of individual PLFA acting on litter decomposition in different habitats and different decomposition periods. Then, we used one-way ANOVA in the case of normal distribution of data and nonparametric test in the case of non-normal distribution of data to compare the differences of individual PLFA concentrations in different decomposition periods of the same habitat and between different habitats in the same decomposition period. We used standard linear regressions to investigate correlations between the concentrations of microbial PLFAs and the ratios of biomarkers and leaf litter mass loss.

3. Results

3.1. Microbial Total Biomass and Community Composition

Total PLFA concentrations, a proxy for microbial biomass, increased significantly ($p < 0.05$) in the nature forest floor, the intermittent stream and the headwater stream from the 28th day of decomposition to the 119th day. The total PLFA concentrations in the headwater stream were significantly higher than those in the intermittent stream and the natural forest floor during the 62nd day of decomposition. However, the total PLFA

concentrations decreased greatly on the 95th day of decomposition in both the intermittent stream and the headwater stream, which made habitat type don't significantly affect the concentrations of total PLFA at the end of litter decomposition (Figure 1a).

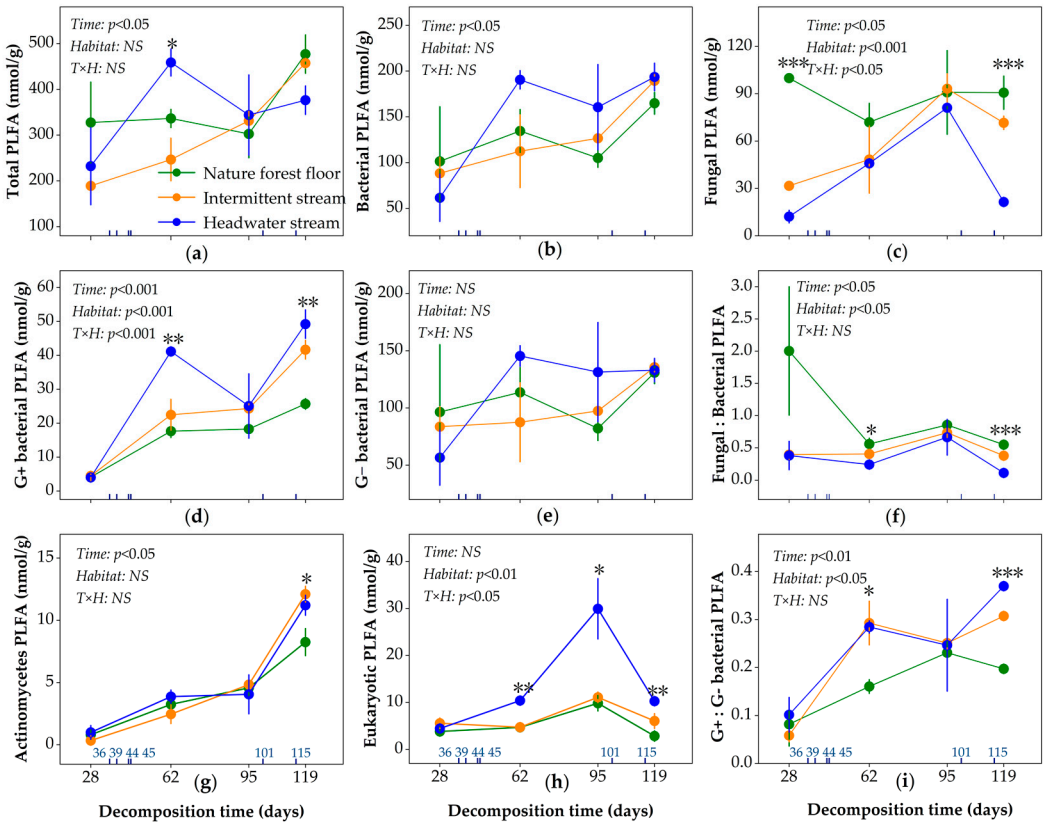


Figure 1. The concentrations of microbial total PLFA (**a**) and PLFA of microbial groups (**b**–**e**,**g**,**h**) in the natural forest floor, intermittent stream, and headwater stream leaf litter and the ratios of biomarkers (**f**,**i**). The blue font indicates the number of days the intermittent stream was dry. The *p* values showed the results from repeated measures ANOVA testing for the effect of habitat type over time. The expression data were shown as mean ± SE. *NS*: not significant. Asterisks denote significant differences among habitat types: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Some microbial community compositions varied over time, and this variation showed different patterns among habitat types. In three habitats, the G+ bacterial and actinomycetes PLFA and the ratios of G+:G− bacteria PLFA (G+/G−) peaked on the 119th day and its concentrations and ratios in the stream and the intermittent stream were significantly higher than that in the nature forest floor. Meanwhile the G+ bacterial PLFA concentrations and the ratios of G+/G− peaked on the 62nd day in the stream (Figure 1d,g,i)). At this point, the G+ bacterial PLFA concentrations in the headwater stream were significantly higher than those in the other two habitats. The ratios of G+/G− in the headwater stream and the intermittent stream were significantly higher than that in the nature forest floor. In the nature forest floor, the fungal PLFA concentrations were significantly higher than those in the other two habitats during the early and late stages of decomposition (Figure 1c). The ratios of fungal: bacterial PLFA (F/B) had a similar trend as the fungal PLFA (Figure 1f). In the headwater stream, the eukaryotic PLFA concentrations were significantly higher than those in the other two habitats during the late stages of decomposition (Figure 1h).

3.2. Distribution Patterns of PLFA

We used the PCA to illustrate the differences among the three habitats for selected PLFAs related to litter decomposition (Figure 2 and Table 1). The PCA performed on the

composition of PLFAs showed that 61.0% and 13.2% of variations were explained by the first and second axes respectively. The total biomass, bacteria, G+ bacteria, G− bacteria, and actinomycete had positive loadings on the PCA-1 in the three habitats. However, fungal and eukaryotic PLFA was unspecific in three habitats. The fungal PLFA had positive loadings on PCA-1 in the intermittent stream and headwater stream and had positive loadings on PCA-2 in the nature forest floor. The eukaryotic PLFA had positive loadings on PCA-1 in the headwater stream and had positive loadings on PCA-2 in both intermittent stream and headwater streams.

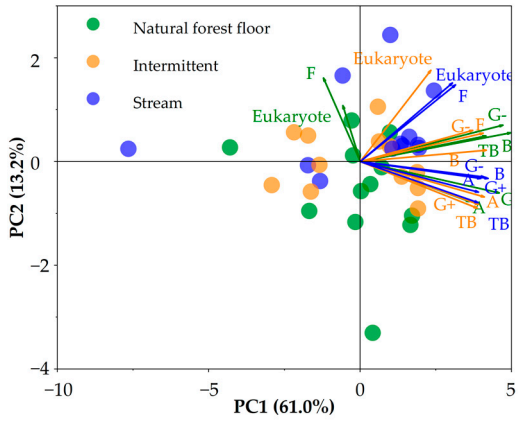


Figure 2. Principal components analyses of PLFA profiles (log_{10} transformed mol) on litter at three habitats. PLFAs in green, orange and blue font represent the natural forest floor, the intermittent stream and stream samples, respectively. TB: Total Biomass, B: Bacteria, F: Fungi, G+: G+ bacteria, G−: G− bacteria, A: Actinomycete.

Table 1. Linear regression for three habitats between the first and second principal components (PCA-1, PCA-2) after the PCA and the concentrations of microbial PLFA. Asterisks denote significant influences of the concentrations of microbial PLFA on the data variation represented by PCA-1 and PCA-2: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Index	PCA-1			PCA-2		
	Natural Forest Floor	Intermittent Stream	Headwater Stream	Natural Forest Floor	Intermittent Stream	Headwater Stream
Total Biomass	0.467 **	0.703 ***	0.816 ***	0.030	−0.030	−0.027
Bacteria	0.820 ***	0.803 ***	0.943 ***	0.039	−0.011	−0.089
Fungi	0.002	0.851 ***	0.337 *	0.035	0.018	0.520 **
G+ bacteria	0.716 ***	0.727 ***	0.882 ***	0.141	−0.069	−0.100
G− bacteria	0.723 ***	0.612 **	0.866 ***	0.143	−0.029	−0.085
Actinomycete	0.366 *	0.816 ***	0.851 ***	0.378	−0.084	−0.079
Eukaryote	−0.086	0.232	0.376 *	−0.098	0.322 *	0.383 *

3.3. Individual PLFA Analysis

The comparison of individual PLFAs in terms of the whole decomposition period considered revealed a major change in the composition of the microbial communities in the three habitats. We identified more individual PLFAs in the natural forest floor and the intermittent stream over the decomposition of the litter (Figure 3). Among them, the concentrations of individual PLFA indicative of G+ bacteria (i15:0, a15:0) increased steadily with decomposition time in the forest floor and the intermittent stream. The concentrations of PLFA indicative of G− bacteria (18:1 w5c) fluctuated greatly with decomposition time (Figure 3a,b). In contrast, there were only two PLFAs (n19:0, 18:2 w6c) in the headwater stream (Figure 3c).

Under the same decomposition period, we identified more individual PLFAs in the later stage of decomposition (Figure 4). The individual PLFA differed more markedly among the three habitats with the increase of decomposition time. In the early stage of decomposition, the difference of individual PLFA was mainly reflected between the forest floor and the aquatic system (Figure 4a,b). There was insignificant difference between

the headwater stream and the intermittent stream. On the contrary, in the later stage of decomposition, the difference of individual PLFA was mainly reflected in the headwater stream and the intermittent stream (Figure 4c,d).

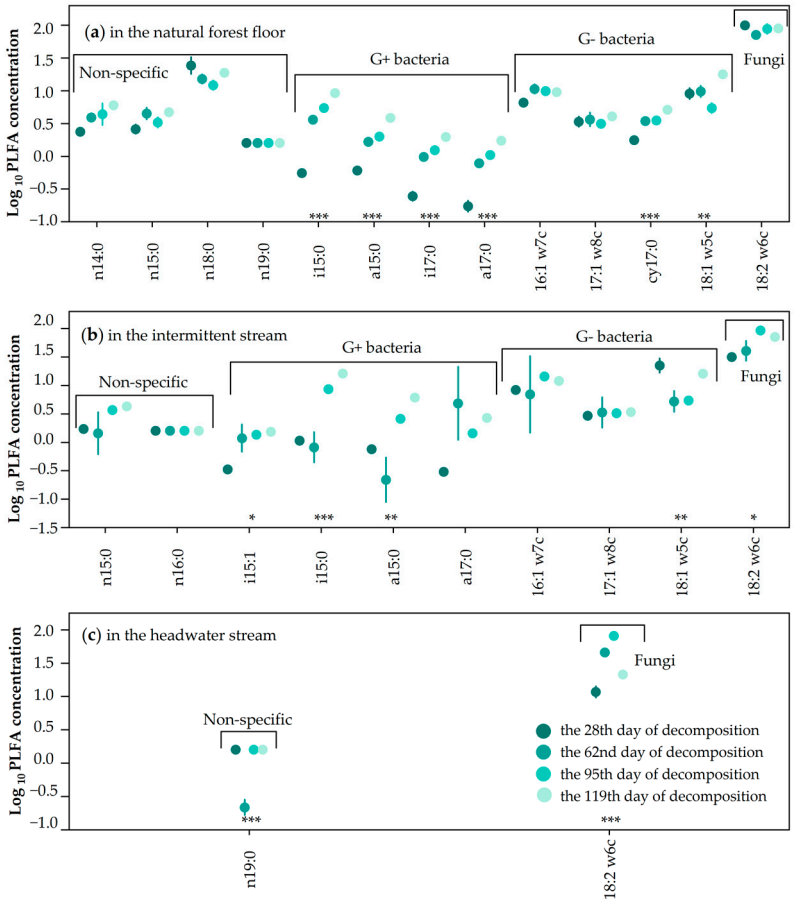


Figure 3. Concentrations of individual PLFA in (**a**) the natural forest floor and (**b**) the intermittent stream and (**c**) the headwater stream leaf litter. Asterisks indicate significant differences among decomposition time: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Dots indicate the mean and whiskers the standard error.

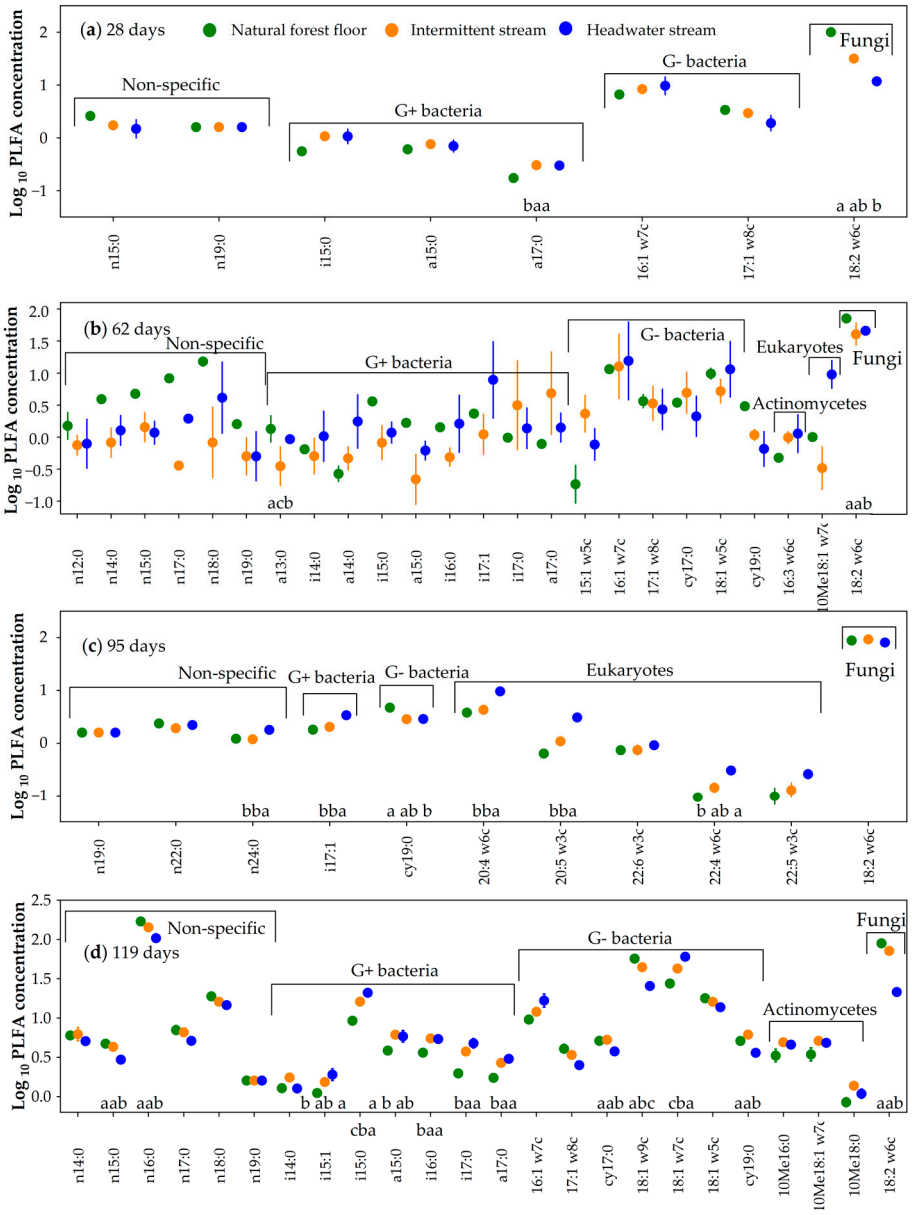


Figure 4. Concentrations of individual PLFA on (**a**) the 28th day of decomposition and (**b**) the 62nd day of decomposition and (**c**) the 95th day of decomposition and (**d**) the 119th day of decomposition leaf litter. Letters indicate significant differences among transect regions ($p < 0.05$). Dots indicate the mean and whiskers the standard error.

3.4. PLFA Biomass and Litter Decomposition

Total PLFA did not correlate with mass loss in three habitats ($p > 0.05$) (Figure 5a). However, the communities of microbial decomposers showed a strong correlation with litter mass loss in three habitats. Among them, the PLFA concentrations of microbial groups and the ratios of biomarkers showed the highest correlation with mass loss in the headwater stream (Figure 5). Specifically, the concentrations of bacterial PLFA, fungal PLFA, G+ bacterial PLFA, G− bacterial PLFA, eukaryotic PLFA, and the G+/G− ratios were related to the loss of litter quality ($p < 0.05$) (Figure 5b,c,e–g,i)). However, only the concentrations of G+ bacterial PLFA, actinomycetes PLFA, and the G+/G− ratios correlated with mass loss in the natural forest floor ($p < 0.001$) (Figure 5d,e,i)). Only the G+/G− ratios correlated with mass loss in the intermittent stream ($p < 0.05$) (Figure 5i).

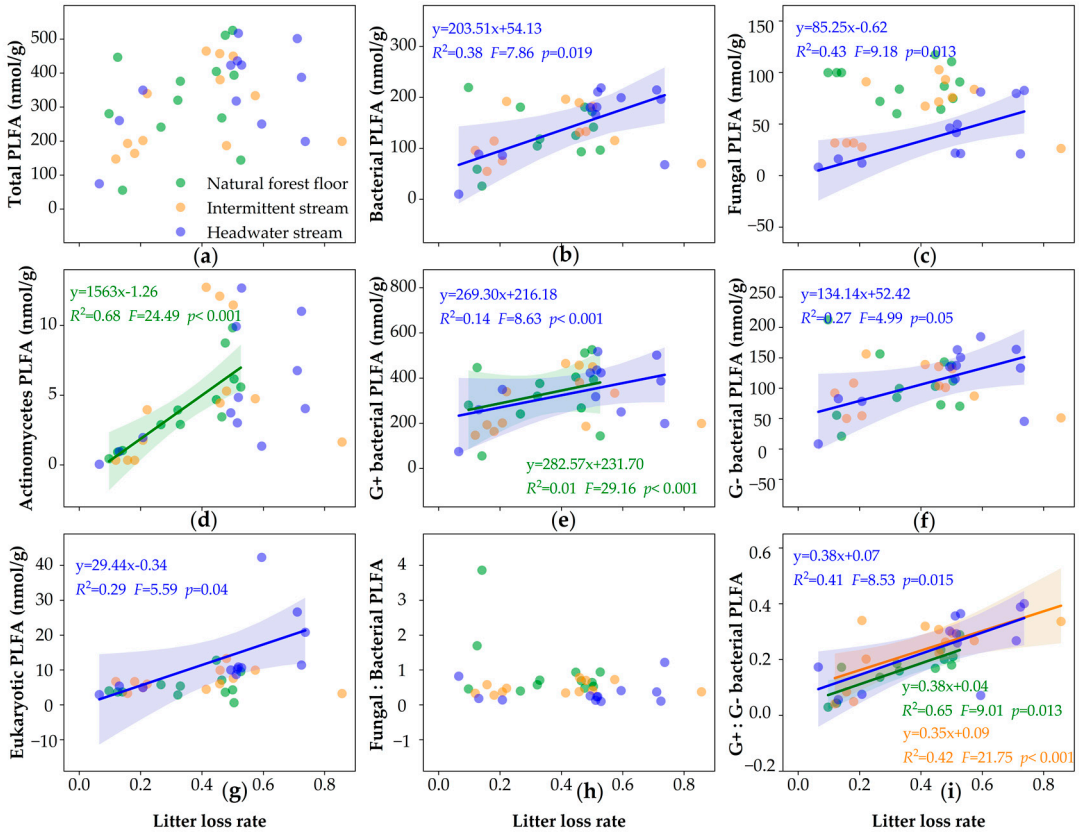


Figure 5. Linear regression for three habitats between litter mass loss and the concentrations of microbial total PLFA (**a**) and PLFA of microbial groups (**b**–**g**), and the ratios of biomarkers (**h**,**i**).

4. Discussions

We analyzed how the microbial community changes during litter decomposition in the natural forest floor, intermittent stream, and headwater stream by PLFA analyses. We found that habitats didn't affect total PLFA concentrations but strongly influenced microbial community composition during the litter decomposition. Meanwhile, microbial total PLFA increased significantly with time among habitat types. Some specific microbial groups showed different trends with decomposition time in three habitats. Meanwhile, linear regression analysis between PLFA concentration and different environmental variables in the three habitats showed that some PLFA were significantly affected by environmental factors (Table 2). For example, bacteria were significantly affected by temperature and pH while fungi didn't show the same pattern. In general, fungi are assumed to be less sensitive to changes in temperature and moisture than bacteria due to chitinous cell walls [3]. In addition, microorganisms ultimately led to direct litter mass loss. Some communities of microbial decomposers such as bacteria (G+ and G− bacteria), fungi, and eukaryote showed a strong correlation with litter mass loss in the headwater stream, which verifies our hypothesis. This indicated that the environmental differences among habitats will affect the development of microbial communities, and ultimately alter litter decomposition [28,29].

Table 2. Linear regression for three habitats between environmental factors (Temperature, Rainfall, pH, Flow rate) and the concentrations of microbial PLFAs.

Microbial Composition	Environmental Factor								
	Temperature			Rainfall			pH		
Natural forest floor	R^2	F	p	R^2	F	p	R^2	F	p
Total Biomass	0.16	3.11	>0.05	0.01	1.16	>0.05	-	-	-
Bacteria	0.03	1.29	>0.05	−0.09	0.12	>0.05	-	-	-
Fungi	−0.09	0.11	>0.05	0.10	2.18	>0.05	-	-	-
G+	0.85	64.8	<0.001	0.01	1.05	>0.05	-	-	-
G−	−0.07	0.27	>0.05	−0.10	0.01	>0.05	-	-	-
Actinomycetes	0.84	56.7	<0.001	0.25	4.66	>0.05	-	-	-
Eukaryote	−0.09	0.12	>0.05	−0.10	0.01	>0.05	-	-	-
Intermittent stream	R^2	F	p	R^2	F	p	R^2	F	p
Total Biomass	0.71	27.3	<0.001	0.26	4.71	>0.05	0.44	9.48	<0.05
Bacteria	0.46	10.5	<0.01	0.17	3.18	>0.05	0.14	2.75	>0.05
Fungi	0.39	7.98	<0.05	0.09	2.13	>0.05	−0.10	0.01	>0.05
G+	0.87	73.1	<0.001	0.10	2.58	>0.05	0.57	15.47	<0.01
G−	0.20	3.68	>0.05	0.11	2.34	>0.05	−0.02	0.83	>0.05
Actinomycetes	0.83	53.6	<0.001	0.41	8.75	<0.05	0.28	5.28	<0.05
Eukaryote	0.01	1.03	>0.05	0.06	1.67	>0.05	0.03	1.32	>0.05
Headwater stream	R^2	F	p	R^2	F	p	R^2	F	p
Total Biomass	0.08	0.84	>0.05	0.01	1.02	>0.05	−0.01	0.93	>0.05
Bacteria	0.33	0.51	<0.05	−0.10	0.01	>0.05	−0.13	0.10	>0.05
Fungi	0.06	1.64	>0.05	−0.09	0.12	>0.05	−0.01	1.10	>0.05
G+	0.43	9.43	<0.05	−0.10	0.02	>0.05	0.46	7.69	<0.05
G−	0.18	3.43	>0.05	−0.09	0.08	>0.05	−0.13	0.10	>0.05
Actinomycetes	0.62	19.1	<0.001	0.22	4.08	>0.05	0.32	4.77	>0.05
Eukaryote	0.15	2.93	>0.05	−0.06	0.43	>0.05	−0.04	0.73	>0.05

The sensitivity of microorganisms to the environment is different, which is greatly reflected in our results. First, the total PLFA concentrations in the intermittent stream and the natural forest floor were significantly lower than that in the headwater stream during the 62nd day of decomposition. This may be that we observed two transient droughts in intermittent streams between days 28th and 62nd of decomposition. At this time, the litter was exposed to solar radiation. The disappearance of water means great physiological stress to microbial decomposers and results in marked decline of its biomass [30]. Moreover, some differences of microbial communities in the three habitats are mainly reflected in fungi, G+ bacteria, and eukaryotes. First, the fungal PLFA concentrations in the natural forest floor were significantly higher than those in other habitats. However, fungal PLFA concentrations were little difference between the headwater stream and the intermittent stream during the whole decomposition period. It is generally accepted that fungi are the main decomposers of refractory substances such as lignin [31]. Once entrained in the ecosystems, litter is rapidly colonized by microorganisms [14]. However, under the conditions of headwater streams and intermittent streams during flow, leaf litter was more vulnerable to leaching [32], which may reduce the colonization of fungi acting on lignin. Some research results also showed that water flow is related to the growth and activity of fungi [17]. Hypoxia conditions in streams may limit the respiration of fungal organisms and inhibit mycelial growth [30]. Meanwhile, the research showed that intermittent stream should be dominated by fungal species with traits of higher desiccation resistance [33]. This makes the fungal concentrations of the intermittent stream similar to that of the headwater stream even under drought. Second, the difference in bacterial concentrations among habitats is mainly reflected in G+ bacteria. Some studies associate G+ bacterial PLFA with stress conditions found that G+ bacteria may be dominant in the condition that the available C is relatively less [34]. Thus, the higher G+ bacterial PLFA in the headwater stream and the intermittent stream suggested lower concentrations of available C received by aquatic ecosystems from land [35]. Meanwhile, the difference in ratios of biomarkers among habitats also showed the different flow of energy and nutrients through the microbial community in different ecosystems [36,37].

Total PLFA concentrations increased significantly with time among habitat types. This may be that some microbial groups (including bacteria, G+ bacteria and actinomycete) also increased significantly with decomposition time in the three habitats. Although the PLFA

concentrations of fungi and eukaryotes decreased significantly at the end of decomposition, their concentrations also increased on the whole. It is generally accepted that fungal biomass increases exponentially, and it is later stabilized or even decreases [38]. However, bacteria play a predominant role in the later stages of litter decomposition [39]. Our results also verify this statement. In addition, Otaki et al. [40] also found similar results in a three-year litter decomposition study. Their study found that the bacterial biomass increased steadily until the end of the experiment while fungal biomass reached its peak in the first year. In a four-month study (from the late summer to the early winter periods), Wilkinson et al. [41] found that bacterial and fungal biomass increased through time on spruce litter in Germany, which is also consistent with our results.

The analysis of individual PLFAs in terms of the whole decomposition period considered revealed a major change in the composition of the microbial communities in the three habitats. It showed that compared with stream conditions, similar strains acting on litter decomposition were more concentrated on the forest floor and intermittent stream. It has been reported that the decomposition environment of stream litter is being influenced by more complex factors such as water chemistry, temperature, velocity, and turbulence, which may result in less individual PLFAs in the whole decomposition period [42]. Under the same decomposition period, we identified more individual PLFAs in the later stage of decomposition. And in the early stage of decomposition, the number of individual PLFA with significant differences among the three habitats was less. This may be due to the large environmental differences among habitats, which makes different microorganisms act on litter decomposition. What is more, their difference was mainly reflected between the forest floor and the aquatic system. The possible reason was that the environmental difference between the two ecosystems may be mainly reflected in the temperature. Temperature is the main driving factor of microbial activity [42,43]. With the increase of temperature, the water temperature is relatively stable, while the atmospheric temperature fluctuates greatly. This led to the difference in individual PLFA between forest floor and water environment habitat. In contrast, in the later stage of decomposition, the difference in individual PLFA is gradually reflected among the three habitats. It was mainly reflected in the headwater stream and the intermittent stream. During this period, the intermittent stream has been in a dry state for a long time, and the litter is exposed to the soil surface. Temporary stress events, such as high temperatures or desiccation, result in dynamic changes in microbial communities over time [44]. This may explain our results.

Litter decomposition is often thought to be regulated by abiotic factors such as microclimate and chemical quality [45]. However, this view changed considerably with the development of molecular tools. Recent studies showed that soil microbial communities differ substantially over the litter decomposition course and space [46,47]. Spatial variation of a rather basic microbial parameter, such as microbial biomass, can be an important determinant of decomposition [48]. Though we did not find direct significant correlations between total PLFA and litter mass loss in three habitats. However, some communities of microbial decomposers showed a strong correlation with litter mass loss. This suggests that, in addition to abiotic factors and potential nutrient transfer among habitat types, the development of some microbial decomposers also drives the decomposition of litter, which further lead to different litter decomposition rates [49]. Among them, the PLFA concentrations of microbial groups showed the highest correlation with mass loss in the headwater stream. Meanwhile, we found fewer common individual PLFAs in the headwater stream throughout the decomposition. This indicated that the diversity of microorganisms is more abundant in the stream environment. A tremendous diversity of microbes contributes to litter decomposition and interacts in a cross-kingdom functional succession of communities [46]. What is more, linking decomposer identity to decomposition could provide a better understanding of the relationship between microbial community structure and leaf litter decomposition.

5. Conclusions

Our findings suggest that habitat do not affect the total biomass of microorganisms, but microbial community composition is significantly influenced by the habitat microenvironment, which might alter the decomposition rate of litter. Results here implied that the number of individual PLFA in the forest floor and intermittent stream was significantly higher than that in the stream in the whole decomposition period. Compared with the late stage of litter decomposition, the difference of individual PLFA concentrations among the three habitats was reflected in the forest floor and aquatic system in the early stage of litter decomposition, but it was mainly reflected in the headwater stream and the intermittent stream in the late stage of litter decomposition. Interestingly, we linked the relationship between microbial community structure and litter decomposition and found that the microbial community showed the greatest correlation with the litter decomposition rate in streams. In conclusion, our results further clarify the dynamics of microbial communities during the litter decomposition in different habitats.

Author Contributions: Conceptualization, H.G., F.W. and X.Z.; data curation, H.G.; formal analysis, H.G.; funding acquisition, F.W. and D.W.; investigation, H.G., X.Z., W.W., L.Z. and R.W.; visualization, H.G.; writing-original draft, H.G.; writing-review and editing, H.G., F.W. and D.W. All authors have read and agreed to the published version of the manuscript.

Funding: The National Natural Science Foundation of China, grant number 32001965, 32171641 and 32101509 which funded this study.

References

1. Hatton, P.J.; Castanha, C.; Torn, M.S.; Bird, J.A. Litter type control on soil C and N stabilization dynamics in a temperate forest. *Glob. Change Biol.* **2015**, *21*, 1358–1367. [CrossRef]
2. Battin, T.J.; Luyssaert, S.; Kaplan, L.A.; Aufdenkampe, A.K.; Richter, A.; Tranvik, L.J. The boundless carbon cycle. *Nat. Geosci.* **2009**, *2*, 598–600. [CrossRef]
3. Zhao, B.; Xing, P.; Wu, Q.L. Interactions between bacteria and fungi in macrophyte leaf litter decomposition. *Environ. Microbiol.* **2021**, *23*, 1130–1144. [CrossRef]
4. Ramirez, K.S.; Craine, J.M.; Fierer, N. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Glob. Change Biol.* **2012**, *18*, 1918–1927. [CrossRef]
5. Parton, W.; Silver, W.L.; Burke, I.C.; Grassens, L.; Harmon, M.E.; Currie, W.S.; King, J.Y.; Adair, E.C.; Brandt, L.A.; Hart, S.C.; et al. Global-scale similarities in nitrogen release patterns during long-term decomposition. *Science* **2007**, *315*, 361–364. [CrossRef] [PubMed]
6. Garcia-Palacios, P.; Shaw, E.A.; Wall, D.H.; Hattenschwiler, S. Temporal dynamics of biotic and abiotic drivers of litter decomposition. *Ecol. Lett.* **2016**, *19*, 554–563. [CrossRef] [PubMed]
7. Graca, M.A.S.; Ferreira, V.; Canhoto, C.; Encalada, A.C.; Guerrero-Bolano, F.; Wantzen, K.M.; Boyero, L. A conceptual model of litter breakdown in low order streams. *Int. Rev. Hydrobiol.* **2015**, *100*, 1–12. [CrossRef]
8. Bradford, M.A.; Berg, B.; Maynard, D.S.; Wieder, W.R.; Wood, S.A. Understanding the dominant controls on litter decomposition. *J. Ecol.* **2016**, *104*, 229–238. [CrossRef]
9. Yue, K.; Garcia-Palacios, P.; Parsons, S.A.; Yang, W.; Peng, Y.; Tan, B.; Huang, C.; Wu, F. Assessing the temporal dynamics of aquatic and terrestrial litter decomposition in an alpine forest. *Funct. Ecol.* **2018**, *32*, 2464–2475. [CrossRef]
10. Joly, F.X.; Kurupas, K.L.; Throop, H.L. Pulse frequency and soil-litter mixing alter the control of cumulative precipitation over litter decomposition. *Ecology* **2017**, *98*, 2255–2260. [CrossRef]
11. Bradford, M.A.; Warren, R.J.; Baldrian, P.; Crowther, T.W.; Maynard, D.S.; Oldfield, E.E.; Wieder, W.R.; Wood, S.A.; King, J.R. Climate fails to predict wood decomposition at regional scales. *Nat. Clim. Change* **2014**, *4*, 625–630. [CrossRef]
12. Shumilova, O.; Zak, D.; Datry, T.; von Schiller, D.; Corti, R.; Foulquier, A.; Obrador, B.; Tockner, K.; Allan, D.C.; Altermatt, F.; et al. Simulating rewetting events in intermittent rivers and ephemeral streams: A global analysis of leached nutrients and organic matter. *Glob. Change Biol.* **2019**, *25*, 1591–1611. [CrossRef] [PubMed]
13. Fenoy, E.; Pradhan, A.; Pascoal, C.; Rubio-Rios, J.; Batista, D.; Moyano-Lopez, F.J.; Cassio, F.; Casas, J.J. Elevated temperature may reduce functional but not taxonomic diversity of fungal assemblages on decomposing leaf litter in streams. *Glob. Change Biol.* **2022**, *28*, 115–127. [CrossRef] [PubMed]
14. Kuehn, K.A. Lentic and lotic habitats as templets for fungal communities: Traits, adaptations, and their significance to litter decomposition within freshwater ecosystems. *Fungal Ecol.* **2016**, *19*, 135–154. [CrossRef]
15. Larned, S.T.; Datry, T.; Arscott, D.B.; Tockner, K. Emerging concepts in temporary-river ecology. *Freshw. Biol.* **2010**, *55*, 717–738. [CrossRef]

16. Febria, C.M.; Hosen, J.D.; Crump, B.C.; Palmer, M.A.; Williams, D.D. Microbial responses to changes in flow status in temporary headwater streams: A cross-system comparison. *Front. Microbiol.* **2015**, *6*, 522. [CrossRef] [PubMed]
17. Mora-Gomez, J.; Duarte, S.; Cassio, F.; Pascoal, C.; Romani, A.M. Microbial decomposition is highly sensitive to leaf litter emersion in a permanent temperate stream. *Sci. Total Environ.* **2018**, *621*, 486–496. [CrossRef]
18. Schlief, J.; Mutz, M. Leaf Decay Processes during and after a Supra-Seasonal Hydrological Drought in a Temperate Lowland Stream. *Int. Rev. Hydrobiol.* **2011**, *96*, 633–655. [CrossRef]
19. Willers, C.; van Rensburg, P.J.J.; Claassens, S. Phospholipid fatty acid profiling of microbial communities-a review of interpretations and recent applications. *J. Appl. Microbiol.* **2015**, *119*, 1207–1218. [CrossRef]
20. Zelles, L. Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere* **1997**, *35*, 275–294. [CrossRef]
21. Drenovsky, R.E.; Elliott, G.N.; Graham, K.J.; Scow, K.M. Comparison of phospholipid fatty acid (PLFA) and total soil fatty acid methyl esters (TSFAME) for characterizing soil microbial communities. *Soil Biol. Biochem.* **2004**, *36*, 1793–1800. [CrossRef]
22. Yu, G.R.; Chen, Z.; Piao, S.L.; Peng, C.H.; Ciais, P.; Wang, Q.F.; Li, X.R.; Zhu, X.J. High carbon dioxide uptake by subtropical forest ecosystems in the East Asian monsoon region. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, 4910–4915. [CrossRef] [PubMed]
23. Bardgett, R.D.; Walker, L.R. Impact of coloniser plant species on the development of decomposer microbial communities following deglaciation. *Soil Biol. Biochem.* **2004**, *36*, 555–559. [CrossRef]
24. Tonin, A.M.; Goncalves, J.F., Jr.; Bambi, P.; Couceiro, S.R.M.; Feitoza, L.A.M.; Fontana, L.E.; Hamada, N.; Hepp, L.U.; Lezan-Kowalczuk, V.G.; Leite, G.F.M.; et al. Plant litter dynamics in the forest-stream interface: Precipitation is a major control across tropical biomes. *Sci. Rep.* **2017**, *7*, 10799. [CrossRef]
25. Zhao, Y.; Wu, F.; Yang, W.; Tan, B.; He, W. Variations in bacterial communities during foliar litter decomposition in the winter and growing seasons in an alpine forest of the eastern Tibetan Plateau. *Can. J. Microbiol.* **2016**, *62*, 35–48. [CrossRef]
26. Zelles, L.; Bai, Q.Y. Fractionation of fatty acids derived from soil lipids by solid phase extraction and their quantitative analysis by GC-MS. *Soil Biol. Biochem.* **1993**, *25*, 495–507. [CrossRef]
27. Otaki, M.; Takeuchi, F.; Tsuyuzaki, S. Changes in microbial community composition in the leaf litter of successional communities after volcanic eruptions of Mount Usu, northern Japan. *J. Mt. Sci.* **2016**, *13*, 1652–1662. [CrossRef]
28. Gessner, M.O.; Swan, C.M.; Dang, C.K.; McKie, B.G.; Bardgett, R.D.; Wall, D.H.; Hattenschwiler, S. Diversity meets decomposition. *Trends Ecol. Evol.* **2010**, *25*, 372–380. [CrossRef]
29. Kardol, P.; Cregger, M.A.; Campany, C.E.; Classen, A.T. Soil ecosystem functioning under climate change: Plant species and community effects. *Ecology* **2010**, *91*, 767–781. [CrossRef]
30. Foulquier, A.; Artigas, J.; Pesce, S.; Datry, T. Drying responses of microbial litter decomposition and associated fungal and bacterial communities are not affected by emersion frequency. *Freshw. Sci.* **2015**, *34*, 1233–1244. [CrossRef]
31. Arnstadt, T.; Hoppe, B.; Kahl, T.; Kellner, H.; Krüger, D.; Bauhus, J.; Hofrichter, M. Dynamics of fungal community composition, decomposition and resulting deadwood properties in logs of Fagus sylvatica, Picea abies and Pinus sylvestris. *For. Ecol. Manag.* **2016**, *382*, 129–142. [CrossRef]
32. Marks, J.C. Revisiting the Fates of Dead Leaves That Fall into Streams. In *Annual Review of Ecology, Evolution, and Systematics*; Futuyma, D.J., Ed.; Annual Reviews: Palo Alto, CA, USA, 2019; Volume 50, pp. 547–568.
33. Shearer, C.A.; Descals, E.; Kohlmeyer, B.; Kohlmeyer, J.; Marvanova, L.; Padgett, D.; Porter, D.; Raja, H.A.; Schmit, J.P.; Thorton, H.A.; et al. Fungal biodiversity in aquatic habitats. *Biodivers. Conserv.* **2007**, *16*, 49–67. [CrossRef]
34. Bird, J.A.; Herman, D.J.; Firestone, M.K. Rhizosphere priming of soil organic matter by bacterial groups in a grassland soil. *Soil Biol. Biochem.* **2011**, *43*, 718–725. [CrossRef]
35. Cole, J.J.; Prairie, Y.T.; Caraco, N.F.; McDowell, W.H.; Tranvik, L.J.; Striegl, R.G.; Duarte, C.M.; Kortelainen, P.; Downing, J.A.; Middelburg, J.J.; et al. Plumbing the global carbon cycle: Integrating inland waters into the terrestrial carbon budget. *Ecosystems* **2007**, *10*, 171–184. [CrossRef]
36. Hogberg, M.N.; Hogbom, L.; Kleja, D.B. Soil microbial community indices as predictors of soil solution chemistry and N leaching in *Picea abies* (L.) Karst. forests in S. Sweden. *Plant Soil* **2013**, *372*, 507–522. [CrossRef]
37. Bragazza, L.; Bardgett, R.D.; Mitchell, E.; Buttler, A. Linking soil microbial communities to vascular plant abundance along a climate gradient. *New Phytol.* **2015**, *205*, 1175–1182. [CrossRef]
38. Artigas, J.; Gaudes, A.; Munoz, I.; Romani, A.M.; Sabater, S. Fungal and Bacterial Colonization of Submerged Leaf Litter in a Mediterranean Stream. *Int. Rev. Hydrobiol.* **2011**, *96*, 221–234. [CrossRef]
39. Duarte, S.; Pascoal, C.; Alves, A.; Correia, A.; Cassio, F. Assessing the dynamic of microbial communities during leaf decomposition in a low-order stream by microscopic and molecular techniques. *Microbiol. Res.* **2010**, *165*, 351–362. [CrossRef]
40. Otaki, M.; Tsuyuzaki, S. Succession of litter-decomposing microbial organisms in deciduous birch and oak forests, northern Japan. *Acta Oecologica* **2019**, *101*, 103485. [CrossRef]
41. Wilkinson, S.C.; Anderson, J.M.; Scardelis, S.P.; Tisiafouli, M.; Taylor, A.; Wolters, V. PLFA profiles of microbial communities in decomposing conifer litters subject to moisture stress. *Soil Biol. Biochem.* **2002**, *34*, 189–200. [CrossRef]
42. Brown, J.H.; Gillooly, J.F.; Allen, A.P.; Savage, V.M.; West, G.B. Toward a metabolic theory of ecology. *Ecology* **2004**, *85*, 1771–1789. [CrossRef]
43. Chen, Y.M.; Gao, J.T.; Xiong, D.C.; Yuan, P.; Zeng, X.M.; Xie, J.S.; Yang, Y.S. Effects of soil warming on soil microbial community structure and soil available nitrogen in subtropical young Chinese fir plantation. *J. Subtropic. Resour. Environ.* **2016**, *11*, 1–8.

44. Penuelas, J.; Rico, L.; Ogaya, R.; Jump, A.S.; Terradas, J. Summer season and long-term drought increase the richness of bacteria and fungi in the foliar phyllosphere of Quercus ilex in a mixed Mediterranean forest. *Plant Biol.* **2012**, *14*, 565–575. [CrossRef] [PubMed]
45. Huang, J.X.; Huang, L.M.; Lin, Z.C.; Cheng, G.S. Controlling factors of litter decomposition rate in China's forests. *J. Subtropic. Resour. Environ.* **2010**, *5*, 56–63.
46. Herzog, C.; Hartmann, M.; Frey, B.; Stierli, B.; Rumpel, C.; Buchmann, N.; Brunner, I. Microbial succession on decomposing root litter in a drought-prone Scots pine forest. *ISME J.* **2019**, *13*, 2346–2362. [CrossRef]
47. Baldrian, P. Forest microbiome: Diversity, complexity and dynamics. *Fems Microbiol. Rev.* **2017**, *41*, 109–130. [CrossRef]
48. Bradford, M.A.; Veen, G.F.; Bonis, A.; Bradford, E.M.; Classen, A.T.; Cornelissen, J.H.C.; Crowther, T.W.; De Long, J.R.; Freschet, G.T.; Kardol, P.; et al. A test of the hierarchical model of litter decomposition. *Nat. Ecol. Evol.* **2017**, *1*, 1836. [CrossRef]
49. Zhang, X.; Wang, L.; Zhou, W.; Hu, W.; Hu, J.; Hu, M. Changes in litter traits induced by vegetation restoration accelerate litter decomposition in Robinia pseudoacacia plantations. *Land Degrad. Dev.* **2022**, *33*, 179–192. [CrossRef]