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Linking microbial community composition to C loss rates during wood decomposition

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ABSTRACT

Although decaying wood plays an important role in global carbon (C) cycling, how changes in microbial community are related to wood C quality and then affect wood organic C loss during wood decomposition remains unclear. In this study, a chronosequence method was used to examine the relationships between wood C loss rates and microbial community compositions during Chinese fir (*Cunninghamia lanceolata*) stump decomposition. Our results showed that microbial community shifted from fungi-dominated at early stages (0–15 years) to relatively more bacteria-dominated at later stages (15–35 years) of wood decomposition. Fungal phospholipid fatty acid (PLFA) content primarily explained wood C loss rates at early stages of wood decomposition. Fungal biomass was positively correlated with proportions of relatively high-quality C (e.g., O-alkyl C), but bacterial biomass was positively correlated with low-quality C. In addition, fungi appeared to be the dominated community under low wood moisture (<20%) at early stages, but fungal biomass tended to decrease and bacterial biomass increased with increasing wood moisture at later stages. Our findings suggest that the fungal community is the dominant decomposer of wood at early stages and may be positively influenced by relatively high-quality wood C and low wood moisture. Bacterial community may benefited from low-quality wood C and high wood moisture at later stages. Enhanced understanding of microbial responses to wood quality and environment is important to improve predictions in wood decomposition models.

1. Introduction

Forests are estimated to contain 360 Pg carbon (C) in plant biomass, with 10–20% as coarse woody debris (CWD, Brown, 2002; Goodale et al., 2002). Despite the importance of CWD to local and global C budgets, the mechanisms driving CWD fluxes and pools are not fully understood after several decades of research (Bradford et al., 2014). The increase in the frequencies of disturbances such as drought, insect outbreaks, wildfire, and wind damage are predicted to increase the abundance of woody detritus in the future (Cornwell et al., 2009; Woodall et al., 2015). Therefore, a better understanding of CWD decomposition processes will help inform estimates of forest C storage under future climate change scenarios.

Previous studies have shown that climate was thought to be the key determinant of decomposition rates at the global scale, with biotic factors controlling at the local and regional scales (Berglund et al., 2013; Bradford et al., 2014). Biotic factors mainly include decomposing organisms (i.e., wood microbes) and wood quality (e.g., chemical composition) (Cornwell et al., 2009). Woods of higher concentration of nitrogen (N) and labile C are associated

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with more rapid decay rates, compared to woods with higher concentrations of recalcitrant structural C compounds (e.g. lignin) and lower amount of N (Sinsabaugh et al., 1993; Weedon et al., 2009; Pietsch et al., 2014). Recently, some studies highlighted the importance of local controls (e.g., microbial decomposers, soil fertility and wood quality) on prediction of wood decomposition rates (Bradford et al., 2014; van der Wal et al., 2014). Various plant traits have been examined in a number of case studies (Cornwell et al., 2009; Weedon et al., 2009; Pietsch et al., 2014), but there have been very few studies to explore the relationship between microbial community composition, function and wood quality in different stages of wood decomposition.

Microbial community composition is increasingly considered a key determinant of ecosystem functioning (Fukami et al., 2010). In the processes of wood decomposition, resource competition among microbes may lead to community shifts (Boddy, 2001; Valentin et al., 2014) and influence the decay rate (van der Wal et al., 2014). In general, decomposition rates are largely controlled by the specific composition of fungi and bacteria, and bacteria often have an *r*-selected strategy for C and nutrient use, while fungi are dominated by a *K*-selected strategy (Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2008). Some previous studies have confirmed that N limitation is a serious constraint controlling microbial community on wood decomposition (Cornwell et al., 2009). With differences in life-history traits, fungi with slow biomass turn-over are thought to be competitive at high litter C:N ratios, while fast-growing bacterial *r*-strategists are efficient at low litter C:N (Kaiser et al., 2014). Another important trait for microbes is the ability to release extracellular enzymes to breakdown complex substrates into compounds small enough for uptake (Hoppe et al., 2015). The structure and quality of wood C changes throughout decomposition as more labile fractions become depleted and more recalcitrant C compounds remain (Hoppe et al., 2015). Microbial decomposer communities can change during wood decomposition due to biotic interactions and shifting substrate quality (Rajala et al., 2012). If wood C quality is a primary driver of wood microbial community composition, microbial community composition will shift and in conjunction with shifting decomposition rates during wood decomposition.

Substrate quality may affect bacterial and fungal abundance and then microbial community functioning (Kaiser et al., 2014). For example, within litter decomposition, bacterial decomposers support high turnover rates of easily available substrates, while slower fungal-dominated decomposition pathways support the degradation of more complex organic material (Wardle et al., 2004; Bray et al., 2012). If there is a similar relationship between microbial community composition and substrate quality during wood decomposition, a greater proportion of high quality substrates will tend to have a lower ratio of fungal:bacterial biomass at early stages than those with low quality substrates at later stages. Indeed, fungi have a higher competitive advantage with the ability to produce enzymes and degrade recalcitrant ligno-cellulose complexes than bacteria during wood decomposition (Boddy, 2001; Hoppe et al., 2015). Environmental conditions also influence the composition of the decomposer community, especially fungi (A'Bear et al., 2014). As wood decomposition proceeds, wood polymer structures are degraded, which increases wood moisture content and microbial access to wood and thus may affect microbial community composition (Hoppe et al., 2015). It is expected that shifts of microbial community composition can directly influence wood decomposition because microbial communities likely vary in their functional traits with changes of substrate quality and environmental conditions (Rajala et al., 2012; van der Wal et al., 2014). Therefore, it is critical to explore the changes in microbial decay community related to substrate characteristics and environmental factors throughout CWD decomposition stages.

We examined the effects of substrate quality of dead wood and environmental conditions on microbial community composition and functioning in a chronosequence of Chinese fir (*Cunninghamia lanceolata*) stumps in the first 35 years of decomposition. A field experiment was established to test two specific hypotheses: (H1) the relative abundance of bacteria will benefit from high C quality more than that of fungi at early stages of wood decomposition; and (H2) wood decomposition rates are mainly determined by bacterial community at early stages.

2. Materials and methods

2.1. Site description and field sampling

A stump decomposition experiment was conducted at Xiayang forest farm (26°48′N, 117°58′E), northwest Fujian Province, South Eastern China. The soil and climate information was described in our previous publication (Hu et al., 2014). The site has a deep red soil classified as a sandy clay loam Ferric Acrisol according to the FAO/UNESCO classification. The climate is humid subtropical monsoon with a mild winter in January and February, and a hot and humid summer between June and October. Spring and autumn are warm transitional periods. Mean annual precipitation and temperature were 1653 mm and 19.5 °C, respectively, with most rain events occurring in spring and summer.

At the site, five one hectare plots were chosen where Chinese fir plantations had been clear-cut approximately 0, 2, 5, 15 and 35 years prior to the study. Before Chinese fir harvesting, the plots were pure mature Chinese fir stands with an age of approximately 25 years on hill slopes (230–278 m elevation). Plots were situated next to each other on the same hillside and had similar slope and elevation. All plots had similar soil texture (Huang, 2013). After harvesting, plots were planted with Chinese fir seedlings at 2 m × 2 m spacing to make up 2500 stems ha^{-1}. Stump samples were collected in July 2013, more than one month after the last rainfall based on weather records. This ensures that wood moisture content was not affected by recent rainfall events. We randomly sampled 15 evenly distributed stumps in plots from each age class (diameter 25–30 cm, height 20–30 cm), but only 5 stumps in the 35-year age class due to limited stump availability. Stump discs were obtained 5 cm from the top using a chain saw, axe and knife. Discs were transported in an icebox to the laboratory where they were stored at −20 °C for less than 2 days prior to the processing. At the time of stump sampling, three soil cores (2.5 cm in diameter, 0–10 cm deep) were randomly collected around each stump. Each bulked soil sample was thoroughly mixed. After stones and visible roots were removed, the soil was sieved (<2 mm) and stored at field moisture content at 4 °C until analysis, usually within 2 days following sampling.

2.2. Wood density and moisture content analyses

For each disc, a wedge-shaped piece (1/8 of the total disc) was removed. The total mass of each segment was weighed on a portable electronic scale (Ohaus Model CT6000), and its volume was determined gravimetrically by water displacement. All samples were then oven dried at 75 °C to a constant mass before measurement of dry mass. Density of each segment was calculated as dry mass per unit volume (g/cm^3), and moisture content (%) was calculated as ((mass wet wood - mass dry wood)/dry wood) × 100%.

2.3. Wood sample preparation

Sawdust samples were taken from each stump disc using an electric drill (bit diameter 6 mm). At least 25 drilled holes were made in each disc and the drill bit was sterilized between samples with ethanol. The bark, if still present, was ground and mixed in 1/8 proportion (the percentage of total hole areas to disc area in each disc) of its area with wood. The area proportion (in %) of bark was visually estimated at different years of decomposition (Shorohova et al., 2012), with 100%, 90%, 75%, 15% and 0% for 0 year, 2 years, 5 years, 15 years and 35 years of decomposition, respectively. The resulting sawdust samples were stored at −20 °C until further analyses.

2.4. Woody C and N density and NMR spectroscopy

The C and N concentrations were determined from the milled subsamples with a LECO CHN-2000 analyser (LECO Corp., St. Joseph, MI). We calculated C and N density ($g \cdot cm^{-3}$), as suggested by Russell et al. (2015), rather than % C or N because using a percentage may be confounded by changes in wood volume over time. The densities of C and N in the stump were calculated by multiplying the concentrations at time t by the total wood density at time t, which results in a better representation of changes in C or N over time. The C/N ratio was calculated as C concentration divided by N concentration.

We used solid-state ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR) with cross-polarization and magic-angle spinning to assess the C molecular structure during wood decomposition. Stump sub-samples of the replicates in each age class were mixed for solid-state ^{13}C NMR analyses. The NMR spectra were obtained at a frequency of 100.59 MHz on a Varian Unity Inova400 spectrometer (Varian Inc., Palo Alto, CA). Subsamples were packed in a silicon nitride rotor (o.d. = 3.6 μs) and spun at 5 kHz at the magic angle. Single contact times of 0.5 ms were applied, with a recycle delay of 1.5 s. Transients (6400) were collected for all samples and a Lorentzian line broadening function of 20 Hz was applied to all spectra. Chemical shift values were referenced externally to hexamethylbenzene at 132.1 ppm, which is equivalent to tetramethylsilane at 0 ppm. Spectra were divided into seven regions representing different chemical environments of a ^{13}C nucleus. These regions were alkyl-C (0–45 ppm), N-alkyl C (45–60 ppm), O-alkyl-C (60–90 ppm), acetal C (90–110 ppm), aromatic-C (110–145 ppm), phenolic-C (145–160 ppm) and carboxyl-C (160–185 ppm). The relative area of each region (as a percentage of total area) was measured by integration of the spectral regions.

2.5. Wood cellulose and lignin concentration

Subsamples (100 g) from 5 discs per plot (randomly selected) were analyzed for lignin and cellulose concentration following the International Association of Analytical Communities (AOAC International) official Uppsala method (Theander et al., 1995). Briefly, lignin and cellulose were determined gravimetrically in the extract residues after treatment with 72% sulphuric acid and sodium chlorite, respectively. The concentration of lignin was considered as Klasson lignin. Acid detergent fiber (ADF) is largely a measure of the combined cellulose and lignin fraction in plant material. The concentration of cellulose was calculated as ADF minus lignin. The lignin:N ratio was calculated as lignin concentration divided by N concentrations.

2.6. Phospholipid fatty acid extraction

We analyzed microbial community composition in stumps using phospholipid fatty acids (PLFA) from three gram subsamples stored at −20 °C. A detailed description of the PLFA methodology followed can be found in Huang et al. (2013). In brief, polar lipids were extracted from freeze-dried stump samples using a modified extraction process (Huang et al., 2013). Extracts were purified on pre-packed silicic acid columns (Agilent Technologies, Wilmington, DE) before being subjected to mild alkaline methanolysis to form fatty acid methyl esters (FAMEs). Individual FAMEs were identified by gas chromatography (Hewlett Packard 5890 GC, equipped with 6890 series injector, flame ionization detector and an Ultra 2 capillary column; 25 m × 0.2 mm, 0.33 mm film thickness) based on their retention times and in combination with the MIDI Sherlock Microbial Identification System (MIDI Inc., Newark, DE).

Total lipid abundance and microbial biomass were calculated as the sum of lipids with chain lengths from C10 to C20. The PLFAs chosen to indicate bacterial biomass were: all iso and anteiso branch chain fatty acids (Denef et al., 2009), monounsaturated and cyclopropane fatty acids (Frostegård et al., 2011; Ushio et al., 2013). The 18:2ω6,9 and 18:1ω9 were used as an indicator of fungi, while 16:1ω5*c* was used to indicate arbuscular mycorrhizal fungi (Swallow et al., 2009). The PLFAs 10 Me16:0, 10 Me17:0 and 10 Me18:0 were used to indicate actinomycetes. The abundance of individual PLFAs was calculated as the absolute amount of C in wood compounds (nmol PLFA-C g^{-1}). Fungi:bacteria ratios were calculated as ratio of 18:2ω6,9 plus 18:1ω9 to the sum of all bacterial lipids.

2.7. Soil C, N and microbial biomass C content

Total soil C and N contents were determined on finely ground (<0.20 mm) soil samples using a LECO EPS-2000 CNS thermal combustion furnace (LECO Corp., St Jose, MI). Five of the homogenized soil samples per plot were randomly selected for soil microbial biomass C (MBC). MBC content was measured by the fumigation-extraction method (Vance et al., 1987) using a Shimadzu TOC-TN analyser (Shimadzu Corp., Kyoto, Japan).

2.8. Data analysis

Rate constants k for wood decomposition were estimated by fitting a single exponential decay model (Eq. (1)) (Olson, 1963).

$$X_t = X_0 e^{-kt} \tag{1}$$

where X_t is the density of CWD at time t, X_o is the initial density of substrate, and t is the time. This rate constant was then used to estimate the time required for 95% mass loss $T_{0.95}$ as:

$$T_{0.95} = -\frac{\ln(0.05)}{k} = \frac{3}{k} \tag{2}$$

C loss rate was estimated by the formula:

$$R = \frac{\left(1 - \frac{C_t}{C_0}\right)}{t} * 100 \tag{3}$$

where C_0 is the C mass at initial time, C_t is the C mass at t time, and t is the time.

Microbial functional diversity was estimated by the Shannon index 'H' (Southwood and Henderson, 2000).

$$H = -\sum_{i=1}^{n} pi \ln pi \tag{4}$$

where pi is the relative abundance of functional group i in n species.

Statistical analyses were performed using SPSS 16.0 (SPSS. Inc., Chicago, IL, USA). One-way ANOVA was used to determine the effect of decay time on density, moisture content, C and N density, C/N ratio, lignin and cellulose concentration, lignin/N ratio and microbial community composition in wood samples and C, N and MBC concentrations of soil samples. Means were compared using least significant difference (LSD) Duncan test. The probability level used to determine significance was $P < 0.05$.

Principal Component Analysis (PCA) was performed to determine if samples of the same age grouped together based on the composition of their microbial communities in *R* 3.2.3 with *vegan* package (R Development Core Team, 2015). The linear mixed-effects model with *lme4* package was used to test if the composition of the microbial community is related to wood C loss rate, and wood moisture content is related to microbial biomass. Redundancy analysis (RDA) was used to test if the composition of the microbial community as represented by PLFA lipid measurements was related to wood moisture content, density, C and N density and C/N ratio, and to soil C and N concentration. Linear fits of wood C quality and soil chemistry variables were superimposed on RDA graphs to show the direction of maximum correlation with RDA axes.

3. Results

3.1. Wood density and wood C changes

As expected, the wood density of Chinese fir stumps decreased significantly through time (Fig. 1a, $P < 0.001$). The mean density decreased from 0.46 g cm^{-3} to 0.31 g cm^{-3} at year 35 (Fig. 1a), resulting in a 32.6% loss of initial mass within 35 years. The estimated decay rate constant of 0.0125 indicated that the turnover time (95% mass loss) for Chinese fir stumps is 240 years. Wood C density significantly decreased with decay time (Fig. 1b, $P < 0.001$). Over the entire decay period, the C density in stumps decreased from 0.21 g cm^{-3} to 0.11 g cm^{-3} (Fig. 1b), and Chinese fir stumps lost 47.4% of initial C. The N density and concentration significantly increased in the first 2 years of wood decomposition (Fig. 1b and Fig. S1, $P < 0.01$), after that, N density slightly increased (Fig. 1b, $P < 0.05$). Over the decay period, the C to N ratio significantly decreased as a result of decreasing C content (Fig. 1b, $P < 0.001$).

3.2. Wood microbial PLFA composition

Both the content and % mole abundance of individual lipid biomarkers varied significantly with decay time (Fig. 2 and

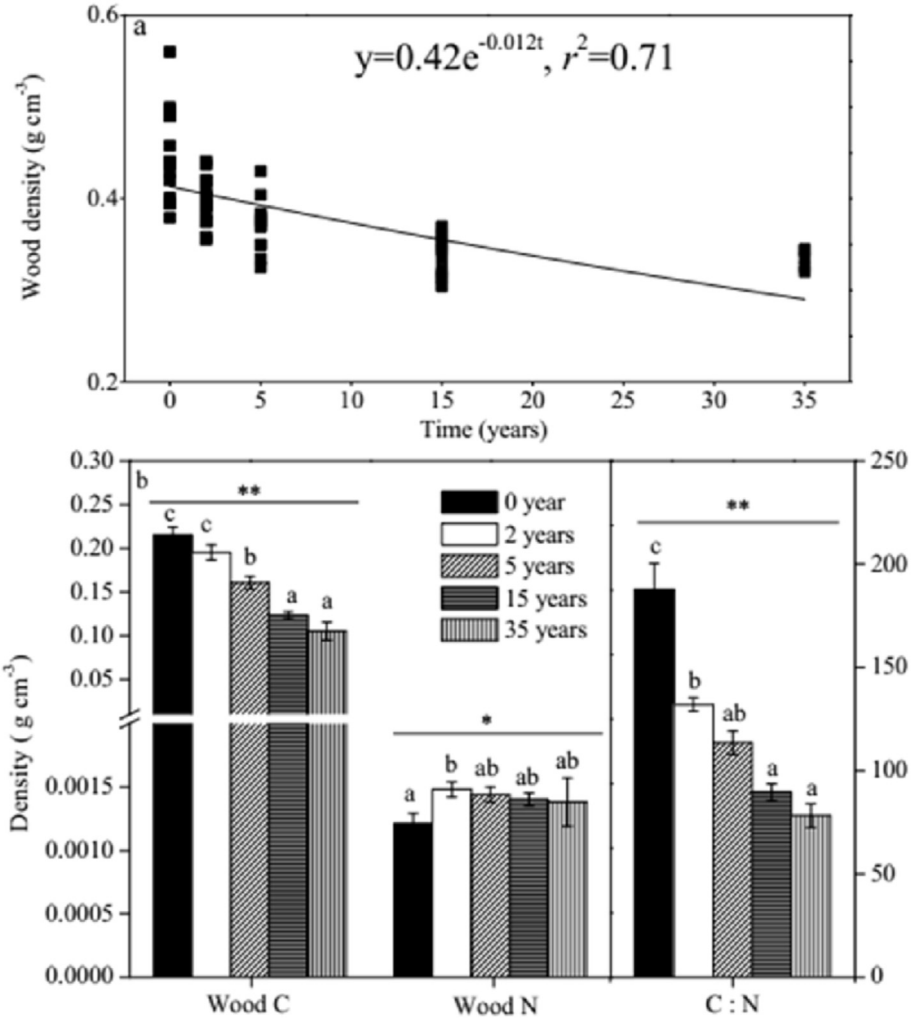


Fig. 1. Changes of wood density, C and N density of Chinese fir stumps from freshly cut to 35 years since cutting. a. Wood density. b. C and N density and C to N ratio. Error bars represent the standard error about the mean, asterisks indicate significant levels at LSD Duncan test (* = $P < 0.05$; ** = $P < 0.01$). Letters identify groups that are significantly different at $P < 0.05$.

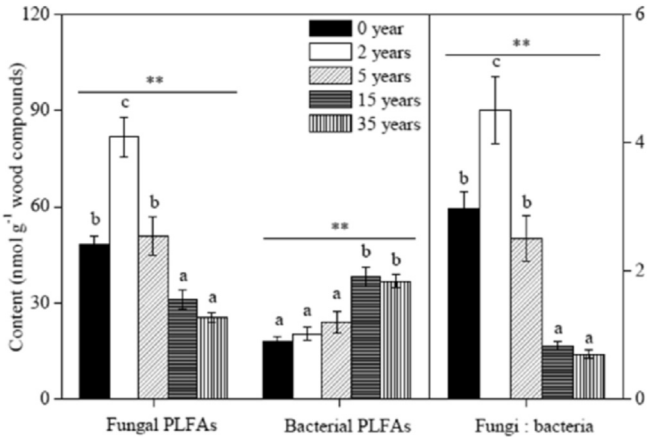


Fig. 2. Mean bacteria and fungi content and fungi:bacteria ratio in the stump wood of Chinese fir after various periods of decomposition. Error bars represent the standard error about the mean, asterisks indicate significance levels at LSD Duncan test (** = $P < 0.01$). Letters identify groups that are significantly different at $P < 0.05$.

Table S1). Fungal PLFAs increased in the first 2 years, and then decreased from year 2 to year 35 ($P < 0.001$). In contrast, the content of bacterial PLFAs significantly increased with the decay time ($P < 0.001$). The wood fungi:bacteria ratio decreased significantly with decomposition time ($P < 0.01$), with a rapid decrease from years 2–35 ($P < 0.05$). Overall, fungi were the dominant taxa within microbial community in the first 15 years, and then bacteria became relatively more abundant after 15 years. Both ANOVA and PCA analysis indicated that microbial community structure differed between the sample times (Table S1 and Fig. S2). The microbial community composition of stump samples in years 15 and 35 were dispersed, while those of years 0, 2 and 5 were clustered together (Fig. S2). These patterns were associated with an increase in microbial diversity in 35 years (Fig. S3).

3.3. C structure by ^{13}C-NMR

^{13}C NMR spectra of stumps samples for different decay times is shown in Fig. S4. Dominant signals were attributed to O-alkyl C which accounted for 35.6–48.4% of total spectra in wood (Table 1). Meanwhile, relative intensity of O-alkyl C signals substantially decreased during wood decomposition. As decomposition proceeded, the relative intensity of signals at alkyl, aromatic and phenolic C increased. We also observed a progressive increase in the ratio of the sum of alkyl, N-alkyl, aromatic and phenolic to the sum of O-alkyl and acetal signals (Table 1).

3.4. Wood C losses in relation to microbial variables

Variation in both fungal PLFAs content and fungi to bacteria ratio were positively related to C loss rates, while variation in bacteria PLFAs was negatively related to C loss rates during wood decomposition (Fig. 3). A significant negative non-linear relation between fungal PLFAs content and the ratio of the sum of alkyl, N-alkyl, aromatic and phenolic to the sum of O-alkyl and acetal signals was observed (Fig. 4a). In contrast, the bacterial PLFAs content was positively correlated with the ratio (Fig. 4b).

3.5. Effects of soil chemistry and wood environment on wood microbial community

RDA indicated separation of microbial communities along the decay profile (Fig. 5), and the first and second axes explained 48.3% and 2.3% of the observed variations in PLFA, respectively. Forward selection in RDA showed that soil N and C, wood moisture and wood N were positively correlated with biomass of most lipid categories associated with bacteria and actinomycetes, while those variables had negative effects on fungal PLFA content. The wood C density and wood density were positive correlated with fungal PLFA content, but they had negative relationship with bacterial PLFA content. Wood moisture content increased with time in this study (Fig. S6). Specially, fungal PLFA content was negatively correlated to wood moisture content during wood decomposition (Fig. 6a), while bacterial PLFA content had a positive relation with wood moisture content (Fig. 6b).

4. Discussion

4.1. Fungi were more competitive than bacteria at early stages

We hypothesized that the bacteria would benefit from high quality of C compounds more than fungi at early stages of decomposition (H1). However, our results indicated that the relative abundance of fungal PLFAs was higher than that of bacterial at early stages (Fig. 2 and Table S1), and the fungal biomass responded to declining quality of wood C compounds through the decomposition process (Fig. 4). We found that fungal PLFAs were over twice as abundant as bacterial PLFAs at years 0, 2 and 5. Priority effects suggest that some fungi may be endophytic and present before tree death, providing them a competitive advantage over other later-arriving microorganisms for available resources (Hiscox et al., 2015). Specifically, this endophyte advantage could promote a fungal dominance of wood compound turnover with easily available C during early decomposition (Boddy, 2001; Rajala et al., 2012). ^{13}C NMR enables quantification of the aliphatic domain of complex macromolecular materials, leading to a more apparent differentiation between lignin C (include alkyl, N-alkyl, aromatic and phenolic C) and polysaccharide C (include O-alkyl and acetal C) during wood decomposition (Preston et al., 1998; Ganjegunte et al., 2004; Bonanomi et al., 2014). Lignin is a complex three-dimensional polymer, which mainly include recalcitrant C compounds (Preston et al., 1998). The polysaccharides (carbohydrates)

Table 1
The relative proportion of different chemical composition (%) within the stump wood of Chinese fir during wood decomposition, which derived from the ^{13}C CPMAS NMR spectra.

Time (years)	Alkyl C	N-alkyl C	O-alkyl C	Acetal C	Aromatic C	Phenolic C	Carbonyl C	Relative proportion[a]
	0–45	45–60	60–90	90-110 ppm	110–145	145–160	160–185	
0	8.9	9.9	48.4	12.7	10.9	4.9	4.4	0.57
2	5.7	10.9	49.5	13.1	11.1	5	4.8	0.53
5	8.6	8.7	48.2	13.1	11.2	5.6	4.7	0.56
15	10.2	10.5	48.1	10.6	12.4	5.9	5.5	0.66
35	12.8	10.8	35.6	11	16.2	7.2	6.4	1.01

[a] (Alkyl + N-Alkyl + aromatic + phenolic)/(O-alkyl + acetal).

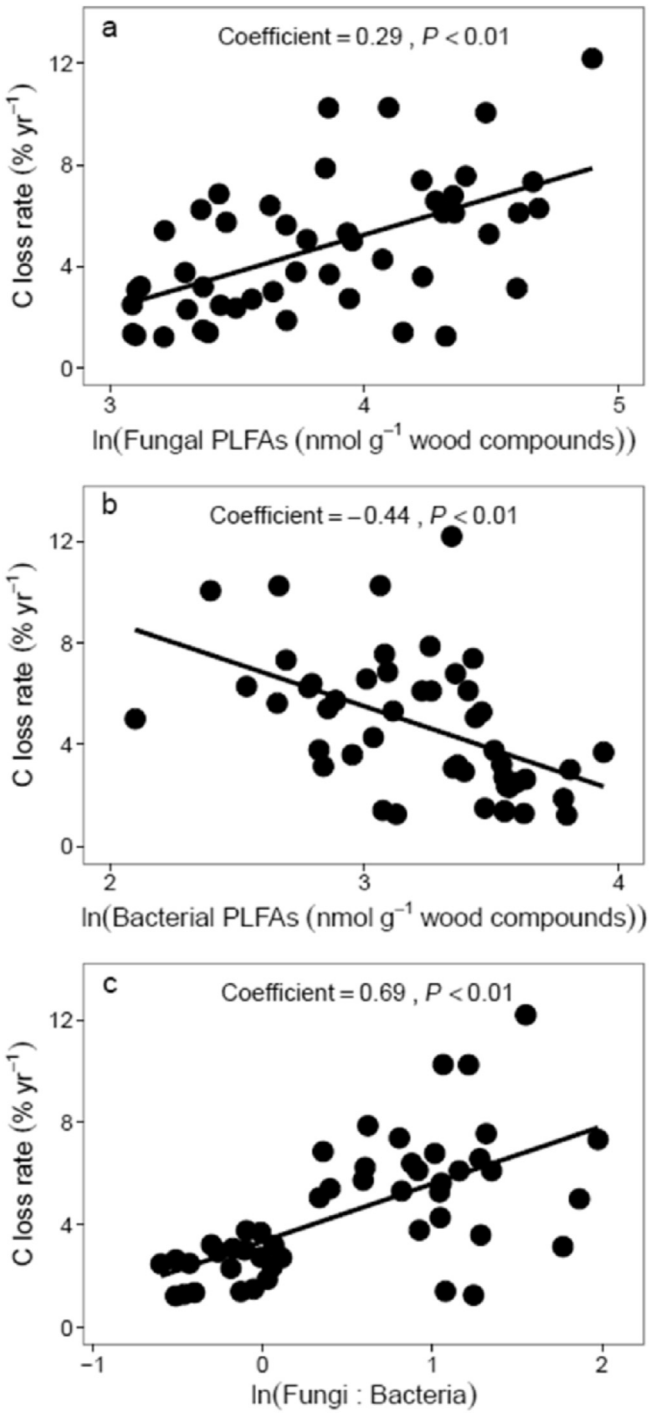
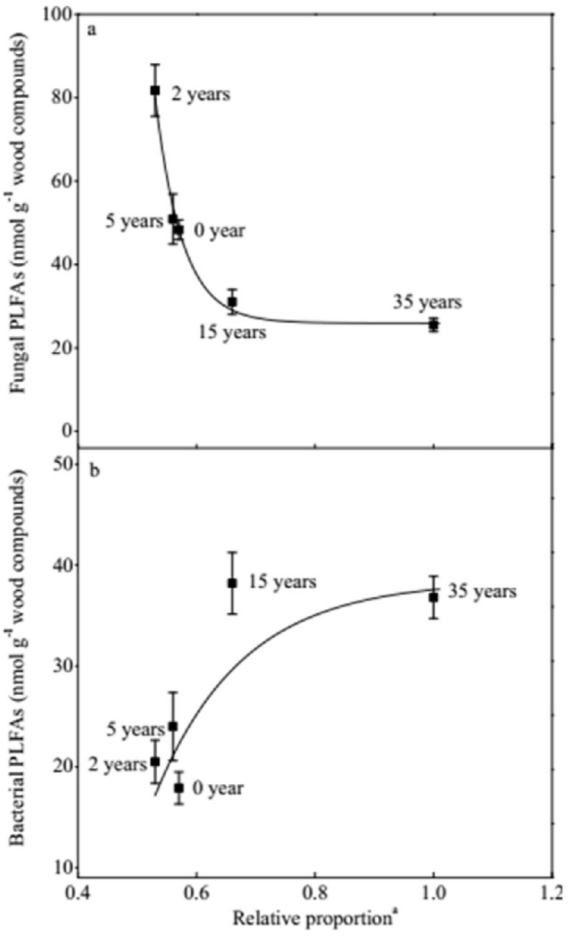


Fig. 3. Relation between C mass loss rate and (a) fungal PLFA content, (b) bacterial PLFA content, and (c) the fungal-to-bacteria PLFA ratio. Data points represent observations for individual stumps (n = 50), regression line show the influence of each variables on C loss rate in the linear mixed model.



[a](Alkyl + N-Alkyl + aromatic + phenolic) / (O-alkyl + acetal).

Fig. 4. The dependence of wood carbon (C) quality (as indicated by ^{13}C NMR) on C groups in (a) fungal PLFAs content ($y = 26 + 6.2e^{-22x}$; $r^2 = 0.98$) and bacterial PLFAs content (b) ($y = 38 - 770e^{-6.8x}$; $r^2 = 0.50$). The fitted relationship is a model standardized major axis regression. Values represent the mean ± SE of five (35 years) or fifteen (other sample times) wood replicates.

include both cellulose and hemicelluloses, which are dominated by relatively high-quality C compounds (Ganjegunte et al., 2004). The ratio of the combined signal intensity of alkyl, N-alkyl, aromatic and phenolic C to the combined signal intensity of O-alkyl and acetal C increased with time during wood decomposition (Table 1), indicating a loss of carbohydrates and accumulation of lignin during wood decomposition. In gymnosperms, primarily white-rot fungi degrade cellulose and lignin early in the process while brown-rot fungi preferentially degraded polysaccharides later (Means et al., 1992; Preston et al., 2012). Meanwhile, we found fungal biomass was strongly correlated with relative high-quality C, and bacteria were linked to recalcitrant C compounds during wood decay (Fig. 4). Rousk and Frey (2015) found that the $\delta^{13}C$ of fungal PLFAs was relatively low during decomposition in the soil, suggesting that fungi used more high-quality organic C than complex C compounds. Furthermore, fungi produce enzymes to degrade wood polymer structures at early stages of wood decay (Stokland et al., 2012), which increases access for bacteria to degrade more recalcitrant compounds later in wood decomposition (Bray et al., 2012). Thus, this study suggests that woody chemistry may play an important role in determining microbial communities throughout the stages of decomposition.

Environmental conditions may have an important effect on microbial community composition (Matulich and Martiny, 2015). Based on previous studies, fungi tend to be more drought-resistant than bacteria during litter decomposition (Bray et al., 2012; Allison, 2012). Wood moisture content was relatively low at initial stages of decomposition (Fig. S6), which could promote growth of fungi over bacteria. In the present study, we found that fungal PLFAs content was negatively related to wood moisture content during

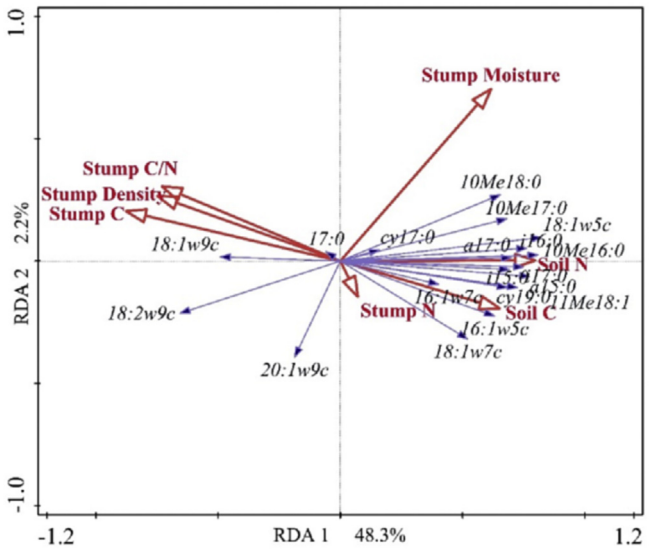


Fig. 5. Redundancy analysis illustrates the separation of microbial communities in decaying Chinese fir wood. These four axes considered significant variables together explained 53.4% of total variation, in which the first axis and second axis explained 48.3% and 2.3%, respectively. In particular, wood C ($F = 38.8$, $P = 0.002$), soil N ($F = 6.1$, $P = 0.002$), wood moisture ($F = 3.0$, $P = 0.004$), wood N ($F = 3.7$, $P = 0.006$) and wood density ($F = 3.3$, $P = 0.05$) contributed 70.7%, 13.1%, 7.3%, 5.6% and 3.2% to the total explained variation.

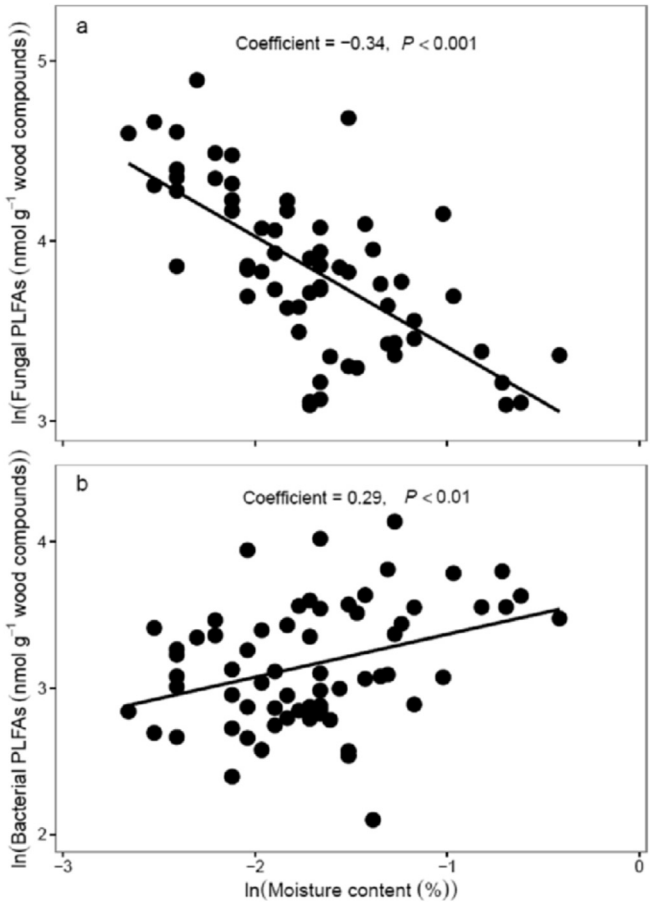


Fig. 6. Relation between wood moisture content and (a) fungal PLFA content and (c) bacteria PLFA content during wood decomposition. Data points represent observations for individual stumps (n = 65), regression line show the influence of wood moisture content on fungi and bacteria biomass in the linear mixed model.

decomposition, while bacterial biomass tended to increase with higher moisture content (Fig. 6). Some litter decomposition experiments showed that fungal growth was greater than bacterial growth in dry environments (Bray et al., 2012; Matulich and Martiny, 2015). As decay proceeded and wood polymers degraded, microbial access points might increase and create a greater range of fungal and bacterial microhabitats at later stages of decomposition (Stokland et al., 2012; Hoppe et al., 2015). RDA indicated that bacterial community abundance was negatively related to wood density and positively related to wood moisture content during decomposition (Fig. 5). Wood pore space and moisture content vary inversely with wood density (A'Bear et al., 2014). Lower wood density may thus lead to higher rates of gas and water exchange at later stages (Cornwell et al., 2009), which could be linked to the adaptation of bacterial groups to the physical environment (A'Bear et al., 2014). The positive correlations of decomposer community abundance with soil C and MBC concentrations suggest that changes in dead wood microbial community composition could be driven by the nutrient supply and microbial colonization from soil (Waldrop and Firestone, 2004).

A technical consideration is that we used the PLFAs 18:2ω6,9 and 18:1ω9 as fungal-specific biomarkers, and our results showed that these fungal biomarkers are greater compared to bacterial PLFA biomarkers in the first 15 years (Fig. 2). Actually, the PLFA 18:2ω6,9 and 18:1ω9 are not exclusive to fungi in the soil microbial community studies, but have also been found to occur in plant tissues (Frostegård et al., 2011; Kaiser et al., 2014). Therefore, the biomass of fungi might be overestimated compared to bacteria (Kaiser et al., 2014). Based on previous studies, plant fatty acids are mainly from root biomass and known to occur in small amounts in plants (Verbruggen et al., 2016). In our study, there are very few roots growing on the surface of stumps, and we removed visible roots prior to analysis. Verbruggen et al. (2016) found that litter-derived ^{13}C retention does not closely track ^{13}C PLFA 18:2ω6,9 measurements, indicating that most fungal PLFAs biomarkers indicate variation in fungi and not plant litter. Although to some extent PLFA 18:2ω6,9 and 18:1ω9 may come from plant tissues, fungal PLFA contents are over two times more than bacterial PLFA content in our study, so their contribution to the total fungal PLFA content in dead wood should be negligible. Therefore, using PLFAs as fungal biomarkers in dead wood may not reduce the reliability of our results.

4.2. Fungi dominate wood C changes during wood decomposition

The decomposition rate observed here ($k = 0.0125$; Fig. 1a) is relatively low compared with the global average level (k value ranged from 0.026 to 0.178, Pietsch et al., 2014), even compared with stump decomposition rates in boreal forests in Finland (Shorohova et al., 2012), but it agrees with values reported for wood decomposition of other conifer species (Harmon et al., 1995; Guo et al., 2006). In addition, our study was carried out on stumps including bark, though we found that almost all the bark was gone at the end of experimental period. Chinese fir deadwood is rich in cyclic dipeptide, a highly active allelochemical that can inhibit microbial decomposers through direct toxicity (Kong et al., 2008). Chinese fir also has a relatively high initial lignin content (32%, Fig. S5) compared to the global mean lignin level in gymnosperms (29.3%, Weedon et al., 2009), which may explain the slower decomposition rates observed here. Lastly, most of the mass in stumps did not directly contact the soil, which is not beneficial to wood decomposition due to lower activity of decomposers and wood moisture (A'Bear et al., 2014).

We hypothesized that bacterial community would be the predominant decomposers on wood C loss at early stages of wood

decomposition (H2). In contrast to H2, our results showed that fungi were the dominant decomposers at early stages. Fungal PLFAs content and fungi:bacteria ratio were positively correlated with C loss rates, while bacterial PLFAs content had a negative correlation during wood decomposition (Fig. 3). This suggests that the decomposition of wood C is primarily affected by the activities of fungal decomposers, rather than bacteria. Fungal colonization is considered a dominant driver of wood mass C loss among microbial communities in CWD (Bradford et al., 2014; van der Wal et al., 2014). In the 35-year decay period, nearly half of C was lost (47.4%, Fig. 1b). The C loss rate was much higher at early stages than that at later stages, a similar to the trend with fungal biomass (Fig. 3). It is generally assumed that fungi have greater ability to generate enzymes than bacteria and thus cause more weight loss at early stages (Bailey et al., 2002; Bray et al., 2012). At later stages, fungi were replaced by bacterial community in community development (Fig. 2). This may indicate that fungi colonized first, resulting in little mass loss (i.e., consuming only the easily available C). Therefore, during resource competition among microbes with low-quality substrates at later stages, fungi may invest more in defensive metabolites than in growth and decomposition (Kaiser et al., 2014).

4.3. Implication for modeling of CWD decomposition

Traditionally, most of wood decomposition models do not explicitly explore microbial community dynamics (e.g. CLM 4.5, LPJmL and CENTURY). Our study provides some insights into applying microbial community dynamics to improve these models. Recently, many studies highlighted the importance of microbial community traits in influencing wood decomposition rates (Bradford et al., 2014; van der Wal et al., 2014; Hoppe et al., 2015). For example, Bradford et al. (2014) found that models parameterized with fungal colonization better predicted observed rates of wood decomposition compared with the reference model without microbial dynamics. Understanding the relative importance and interaction of wood chemistry and environment and microbial variables will help the explicit incorporation of microbial dynamics into wood decay models (Cornwell et al., 2009; Weedon et al., 2009). Our results suggest that fungi dominate the decomposer community for C loss rates at early decomposition stage, and bacteria become relative more abundant than fungi at the late decomposition stage. Because fungal and bacterial communities have very different C use efficiencies (Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2008) and environment condition requirements (Boddy, 2001; Kaiser et al., 2014), our finding could be critical for the development of models that explicitly consider microbial population dynamics. Moreover, understanding of microbial dynamics on wood decomposition is mainly based on foliar litter decomposition and soil organic matter decomposition, leaving wood decomposition as a source of major uncertainty in C-cycle models. With the majority of studies conducted in North American and Western European forests, more data from tropical and subtropical forests around the world would provide more comprehensive understanding for future model development. Explicitly incorporating microbial decomposition of woody debris pools into global vegetation models may improve prediction accuracy of C turnover under current and future climate conditions.

5. Conclusion

Fungi were primary microbial decomposers of wood in the first 15 years of decomposition, but after this period, microbial community structure shifted to relatively more bacteria. The large variation of microbial community composition over time could result from the shift of wood chemical and environmental conditions during decomposition. Our results highlight that fungal groups were strongly influenced by relatively high-quality organic C, but bacterial groups were positively correlated with low-quality C compounds. This was likely due to priority effects for fungal decomposers potentially living as endophytes having an access advantage to the labile C compounds, which could explain why fungi were the dominant decomposers in the decay community at early stages. Moisture content may be an important factor explaining microbial community composition shift, because fungi are more drought-resistant than bacteria at early stages when wood moisture content was relatively lower. Our results highlight that a better understanding of dead wood C processes along with microbial dynamics is essential for improving wood decay models and understanding C pool dynamics in forested ecosystems.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2016.10.017.

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