Differential responses of needle and branch order-based root decay to nitrogen addition: dominant effects of acid-unhydrolyzable residue and microbial enzymes

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Abstract

Background and aims Both chemical differences between foliage and different orders of fine roots and their contrasting decomposing microenvironments may affect their decomposition. However, little is known about how foliage and branch order-based root decomposition responds to increased N availability and the response mechanisms behind.

Methods The effects of different doses of N addition on the decomposition of needles and order-based roots of *Pinus elliottii* (slash pine) were monitored using the litterbag method for 524 days in a subtropical slash pine plantation in south China. The acid-unhydrolyzable residue (AUR) concentration and microbial extracellular enzymatic activities (EEA) in decomposing needles and roots were also determined.

Results Our results indicate that the responses of needle and order-based root decomposition were N-dose-

specific. The decomposition of both needles and lower-order roots was inhibited under the high N dose rate. The retarded decomposition of lower-order roots could be explained more by the increased binding of AUR to inorganic N ions, while the retarded decomposition of needles could be explained more by the reduced microbial EEA. Further, in contrast to lower-order roots, N addition had no effect on the decomposition of higher-order roots.

Conclusions We conclude that the decomposition of foliage and fine roots may fail to mirror each other at ambient conditions or in response to N deposition due to their contrasting decomposition microenvironments and tissue chemistry. Given the differential effects of N addition on order-based roots, our findings highlight the need to consider the tissue chemistry heterogeneity within branching fine root systems when predicting the responses of root decomposition to N loading.

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Introduction

Elucidating the relationships between decomposition of resource-acquiring plant organs (i.e., foliage and fine roots (<2 mm in diameter)) and nitrogen (N) availability is critical for predicting the consequences of increased anthropogenic N deposition to terrestrial ecosystems (Galloway et al. 2004; Hobbie et al. 2012). However, traditional research on these relationships has focused

primarily on the aboveground plant tissues such as foliage (Hobbie 2008; Perakis et al. 2012). Although a growing body of studies suggest that root-derived materials may contribute more to stabilizing soil organic matter than foliage (Rasse et al. 2005; Crow et al. 2009), empirical evidence for the effect of increased N availability on the afterlife of fine roots remains lacking (Conn and Day 1996; Ostertag and Hobbie 1999; Mao et al. 2011; Norris et al. 2013; Solly et al. 2014). This, to a large extent, impedes further understanding of the responses of plant tissue decomposition to increased N availability on a broader scale, and the underlying response mechanisms.

Although both fine roots and foliage turn over rapidly, their decomposition rates may be different under ambient conditions and may even have opposing responses to N additions (Norris et al. 2013). It has been reported that fine roots decompose faster than foliage in N-deficient soils (Ostertag and Hobbie 1999). In addition, a recent study found that N addition stimulates foliage decomposition, but retards root decomposition (Norris et al. 2013). The differential effects of N availability on foliage and fine root decomposition can be ascribed to multiple factors, including microbial decomposer communities, decomposition environment, and tissue chemistry. Compared to foliage, fine roots decompose in the soil where the microbial communities and activities may be well-buffered from climate extremes in temperature and precipitation (Silver and Miya 2001), and from changing environments. Thus, high N loadings may result in fine roots decomposing faster than foliage. Alternatively, fine roots are spatially close to mineral nutrients when compared to foliage (Sanaullah et al. 2011). Hence, the acid-unhydrolyzable residue (AUR, also termed as ‘gravimetric lignin’ that generally includes such components as lignin and phenolics) (Preston et al. 2009; Berg et al. 2013) in decomposing roots may be more liable to adsorb inorganic N ions (Solly et al. 2014) and form compounds highly resistant to microbial degradation (Dijkstra et al. 2004). In addition, fine roots perform totally different functions than foliage, thus they may possess a different chemical composition that plays a pivotal role in subsequent decomposition (Aerts 1997).

Plant tissue chemistry (litter quality) varies not only among organs (Pietsch et al. 2014), but also within organs (e.g., branching fine root systems) (Goebel et al. 2011). For instance, recent studies of foliage and order-based root decomposition have

shown that the distal, short-lived, lower-order roots; (i.e., order 1st and 2nd, generally with finer diameter) within the fine root guild of woody tree species have a higher AUR concentration and thus decompose more slowly than lignified higher-order roots (i.e., order 3rd to 5th, generally with coarser diameter) (Fan and Guo 2010; Xiong et al. 2013) and foliage (Xiong et al. 2013). Given the difference in tissue chemistry, the conventional diameter-based (which may span multiple orders of roots) root decomposition studies may obscure the finer decomposition patterns of roots (Hobbie et al. 2010; Norris et al. 2013) and their decomposition responses to increased N availability. Despite this, less attention has been given to the tissue chemistry heterogeneity within the branching fine root systems, and to the best of our knowledge, no studies have compared foliage and order-based root decomposition responses to N deposition.

In the present decomposition experiment, we focused primarily on the direct effects (i.e., increased N availability, but not modified tissue chemistry) of N deposition, thus all plant material was collected from the unfertilized forests. We monitored and compared the decomposition responses of needles, lower-order roots, and higher-order roots of *P. elliottii* to different doses of N addition (0, 40, and 120 kg N ha⁻¹ yr⁻¹). The ambient wet N deposition level at the site is approximately 10 kg N ha⁻¹ yr⁻¹ with about 68 % reactive N source from NH₄⁺ (Zhan et al. 2014). According to the meta-analysis in Knorr et al. (2005), N addition should exert detrimental effects on litter decomposition when N addition doses are 2–20 times the N deposition level (5–10 kg N ha⁻¹ yr⁻¹), or when litter is rich in lignin (AUR). We therefore hypothesized that (i) exogenous N addition would exert negative effects on decomposition of both needles, due to reduced microbial EEA, and lower-order roots (high initial AUR concentration), due to increased binding of AUR to inorganic N ions. Given the retarded decomposition of needles and lower-order roots, we further hypothesized that (ii) N addition would in turn exert positive effects on decomposition of higher-order roots (relatively low AUR concentration), due to high microbial demand for carbon (C) resources. Finally, we hypothesized that (iii) low- and high-doses of N addition would have contrasting effects on decomposition, since the binding of AUR to inorganic N ions and microbial demand for N would be dose-specific.

Materials and methods

Study site and experimental set-up

The decomposition experiment was conducted at our chronic N-fertilization site at the Qianyanzhou (QYZ) Experimental Station of Red Soil and Hilly Land, Chinese Academy of Sciences (CAS), located in Jiangxi province, southeastern China (26°44′29.1″ N, 115°03′29.2″ E, 102 m a.s.l.). We selected *P. elliotii* (slash pine), one of the dominant species in this region used for vegetation restoration around 1985 (Wang et al. 2012). The study region has a subtropical monsoon climate with mean annual temperature and precipitation of 17.9 °C and 1,475 mm, respectively (Wen et al. 2010). Mean diameter at breast height (DBH) and mean height of the trees are 20.9 cm and 17.5 m, respectively. The weathered soil, from red sandstone and mud stone, is classified to Typic Dystrudepts Udepts Inceptisols (Wang et al. 2012). Soil organic matter content was 58.3 ± 7.5 g kg⁻¹ for 0–5 cm depth and 24.4 ± 2.1 g kg⁻¹ for 5–10 cm depth. The total soil N and P contents were, respectively, 1.7 ± 0.3 g kg⁻¹ and 217 ± 60 mg kg⁻¹ for 0–5 cm depth and 1.0 ± 0.1 g kg⁻¹ and 174 ± 20 mg kg⁻¹ for 5–10 cm depth. The understory vegetation is dominated by *Woodwardia japonica*, *Loropetalum chinense*, and *Dicranopteris dichotoma* (Wang et al. 2012).

A randomized complete block design with three blocks (replicates) was employed. Within each block, three 20×20 m plots (the buffer zone was more than 10 m and the slope angle was <15°) randomly served as a control receiving ambient N deposition, and ambient N deposition plus randomly assigned chronic atmospheric N deposition (40 vs. 120 kg N ha⁻¹ yr⁻¹ as NH₄Cl, respectively). Fertilizers were fully dissolved in 30 L tap water and evenly sprayed onto N-addition plots once per month. The control plots were supplied with equivalent amounts of tap water. The N addition started on 01-May-2012 and proceeded at a month interval on non-rainy days.

Root-derived material and needle preparation

In mid May 2013, intact fine roots were sampled at 0–10 cm depth in the ambient *P. elliotii* forests and transported to the laboratory within 2 hours. The soil and organic matter particles adhering to roots were gently rinsed off with tap water. The clean live and intact roots were hierarchically dissected into branching orders

using fine forceps following the protocol described by Pregitzer et al. (2002). In this study, only the first five order roots were included and partitioned into two types of decomposing substrates. Most distal two order roots (the 1st and 2nd orders) were classified as the lower-order roots and the proximal three order roots (the 3rd to 5th orders) were grouped into higher-order roots. The mean root diameter for the 1st to 5th order was 0.31 ± 0.01 , 0.34 ± 0.01 , 0.42 ± 0.02 , 0.62 ± 0.02 , and 1.26 ± 0.06 mm, respectively. Newly shed needles were collected by hand-picking and then clipped into pieces of approximately 10 cm. The classified roots and needles were first air-dried for storage and then oven-dried to a constant mass at a relatively lower temperature (40 °C) to avoid chemical composition change within tissues.

Litterbag deployment, retrieval, and litter chemical analyses

Root-derived materials were placed into 10×10 cm nylon litterbags with each containing 2.003 ± 0.002 g (dry weight) of lower-order roots or higher-order roots. Both sides of the root litterbag were made of 0.1 mm mesh. Needles were placed into 10×15 cm nylon litterbags with each containing 5.003 ± 0.002 g (dry weight) of needle litter. To match the real decomposition situation, the top side of the needle litterbags was 1 mm meshed and bottom side was identical to root litterbags (i.e., 0.1 mm meshed). Although the mesh size of 0.1 mm would inevitably prevent mesofauna decomposer organisms (e.g., microarthropods) from entering into the litterbags, it was helpful to avoid the physical loss of lower-order roots (>0.3 mm in diameter). Moreover, previous research showed that there were no significant effects of mesh size on the first three root orders when 1 mm mesh was employed (Xiong et al. 2013). On 23-May-2013, one subplot (2.5×3.0 m) was established at the center of each plot. All root litterbags were buried at an angle of approximately 30° to the vertical direction at a depth of 10 cm. Foliage litterbags were placed horizontally and fixed on the ground using steel nails. An additional set of three replicates of each substrate type was assigned for initial tissue chemistry analyses at time of field placement.

Litterbags were retrieved on 31-Jul-2013 (69 days), 29-Nov-2013 (190 days), 28-Feb-2014 (281 days), 29-May-2014 (371 days), and 29-Oct-2014 (524 days). On each sampling date, one litterbag of each substrate type was collected from each subplot. Any litterbags broken

by soil animals or contaminated by pine oil or root exudates would be replaced by a new one for subsequent analysis. At the laboratory, the retrieved litterbags (root litterbags in particular) were processed in accordance with different environmental conditions. When the litterbags were retrieved on the date before which no rainfall events happened (dry litterbags), the litterbags were immediately cleaned by gently brushing exterior soil. However, when the litterbags were retrieved after rain (wet litterbags), all litterbags were first oven-dried, and then attached clay particles were carefully removed prior to opening the litterbags. Additionally, any extraneous material, such as in-growing roots and sometimes tiny arthropods, were carefully removed by forceps. The retrieved litter was oven-dried to a constant weight (40 °C) to determine the dry mass.

All litter samples were ground using a Restch MM400 mixer mill (Retsch GmbH, Haan, Germany) prior to determining the content of C and N using a Vario MACRO cube elemental analyzer (Elementar, Germany). Carbon fractions (extractives, AUR, and acid-hydrolyzable residue, AHR) were determined by a sulfuric acid digestion method combined with chloroform-methanol extraction (Ryan et al. 1990; Xiong et al. 2013). Specifically, a milled powder subsample (200–300 mg) was first extracted with chloroform-methanol ($v/v=2/1$, 15 ml) for 2 h and then filtered. The oven-dried residue (R_1) was digested with sulfuric acid (72 %, 15 ml) for 3 h and then centrifuged (3000 rpm, 8 min). The supernatant in the centrifuge tubes (50 ml) was repeatedly replaced with deionized water (approximately 10 times) until the pH was 7. The residue (R_2) was filtered through a cellulose acetate membrane (0.45 μm) with a vacuum pump and oven-dried to a constant weight (R_3). Ash contents in the sample and in the residue (R_3) were determined by combusting 40–50 mg of sample (550 °C, 4 h). The AUR concentration was calculated as: AUR concentration (%) = $(R_3 - \text{ash-in-}R_3) \times 100 / (\text{subsample} - \text{ash-in-subsample})$. All litter initial chemistry parameters and mass remaining over time were expressed on an ash-free, dry mass basis.

Microbial enzyme activities assay

Upon completion of the decomposition experiment (524 days), one litterbag of each type of decomposing substrate, in each plot, was sampled for microbial EEA. Four hydrolytic enzymes including, β -1,4-glucosidase

(BG, responsible for cellulose degradation), β -1,4-*N*-acetylglucosaminidase (NAG, for chitin degradation), cellobiohydrolase (CBH, for cellulose degradation), acid phosphatase (AP, for organic P mineralization), and one oxidative enzyme (polyphenol oxidase (PPO, for lignin and other aromatic polymers degradation)) were determined following the protocol of Saiya-Cork et al. (2002). One-half of each litter sample was dried at 60 °C to a constant weight to determine water content, and the other half was chopped (approximately 1 cm length for needles and higher-order roots, and original length for lower-order roots) and used (0.5 g) to make sample suspensions with 125 ml of 50 mM acetate buffer (pH 5.0) using a Vortex-Genie 2. The resulting suspensions and pre-prepared acetate buffer, MUB standard, and substrate solution were dispensed into 96-well microplates with eight replicate wells per sample per assay. The analysis involved eight replicate wells for each homogenate control (sample solution + buffer), substrate control (substrate + buffer), and quench standard (sample solution + standard). All the assays except PPO were fluorimetric and the microplates were incubated in the dark at 20 °C for 4 h. To stop the reaction, a 10 μl aliquot of 1 M NaOH was added to each well. Fluorescence was measured using a microplate fluorometer with 365 nm excitation and 450 nm emission filters and a Synergy H4 Hybrid Reader (Synergy^{H4} BioTek, USA). PPO was measured spectrophotometrically using L-3,4-dihydroxyphenylalanine (DOPA) as the substrate and the microplates were incubated in the dark at 20 °C for 18 h. PPO activity was quantified by measuring absorbance at 450 nm. The enzyme activities were expressed in units of $\text{n mol h}^{-1} \text{g}^{-1}$.

Statistical analysis

The single exponential model (Olson 1963) has been demonstrated to work well for various litters before 80 % of the initial mass is lost (Aber et al. 1990; Berg and McLaugherty 2014). Thus, the decay rate constants (k values) in this study were determined by fitting a first-order negative exponential decay equation: $X_t = X_0 e^{-kt}$, where X_t , X_0 , and e are respectively the mass remaining at time t , the initial mass, and the base of natural logarithm.

One-way ANOVA was used to test the differences of the initial tissue chemistry parameters among decomposing substrates. Repeated-measures of ANOVA were used to test the effects of N addition on

mass remaining (of initial), AUR remaining (of initial), N remaining (of initial) of needles and order-based roots with time as a factor. One-way ANOVA was used to test the effects of N addition on microbial EEA. Data were log-transformed where necessary to improve normality and reduce heteroscedasticity. Significant differences between means were compared using Tukey's HSD (Honestly Significant Difference) test. To assess the influences of exogenous, but not native N ions in soil on the increased AUR concentration in decomposing substrates, simple linear regression analyses were conducted to fit net AUR remaining against net N remaining, where the "net remaining" was calculated by deducting the AUR (N) remaining of the control from those of N treatments (the net values at all sampling dates were used, $n=30$). Also, we used simple linear regression analyses to test the relationships between decay rate constants (k) and associated parameters including microbial EEA (BG, NAG, CBH, AP, and PPO) and AUR remaining (the mean values during the course of decomposition) in three types of decomposing substrates (all treatments were pooled, $n=9$). All statistical analyses were performed using the SPSS software version 18.0.

Results

Initial litter tissue chemistry

Initial AUR and N concentrations were significantly higher in lower-order roots than in higher-order roots and needles (Table 1). In contrast, the highest AHR

concentration was found in higher-order roots and the lowest one in lower-order roots (Table 1). AUR: N ratios and extractives were highest in needles, but did not differ between two types of roots (Table 1). Needles and higher-order roots had higher AHR: N ratios than lower-order roots (Table 1). Needles had significantly higher C: N ratios than the higher-order roots and lower-order roots (Table 1). Lower-order roots had higher AUR: AHR ratios and higher-order roots had lower AUR: AHR ratios (Table 1).

Responses of foliage and order-based root decomposition to N addition

During the entire period of decomposition, exogenous N addition exerted differential effects on decomposition of needles, lower-order roots, and higher-order roots, and the effects varied with the dose of N added and over time ($P<0.001$, Fig. 1). Specifically, a high dose of N significantly retarded needle decomposition by 4 % on average ($P=0.027$, Fig. 1a) and lower-order root decomposition by 5 % on average ($P=0.003$, Fig. 1b). In contrast, N addition had no effect on higher-order root decomposition ($P>0.05$, Fig. 1c). Across treatments, the decay rate constant k ranged from 0.216 to 0.246 year⁻¹ in needles, from 0.182 to 0.214 year⁻¹ in lower-order roots, and from 0.273 to 0.280 year⁻¹ in higher-order roots (Table 2).

AUR concentrations and N release patterns

The AUR remaining (of initial) in needles and order-based roots increased over time (Fig. 2a, b, and c). High dose of N significantly increased the AUR

Table 1 Initial tissue chemistry in needles and branch order-based roots of *Pinus elliottii*

Substrate type	C fraction and N concentration				Key ratio			
	Extractives (%)	AUR (%)	AHR (%)	N (mg g ⁻¹)	C:N	AUR:N	AHR:N	AUR:AHR
Lower-order roots	12.14 ^b (0.79)	50.41 ^a (1.22)	37.45 ^c (0.88)	12.96 ^a (0.07)	36.34 ^c (0.21)	38.92 ^b (1.10)	28.90 ^b (0.52)	1.35 ^a (0.06)
Higher-order roots	11.12 ^b (0.76)	36.28 ^b (0.54)	52.60 ^a (0.23)	9.11 ^b (0.11)	50.46 ^b (1.44)	39.86 ^b (0.96)	57.77 ^a (0.91)	0.69 ^b (0.01)
Needles	19.31 ^a (0.50)	36.63 ^b (1.02)	44.06 ^b (0.52)	7.22 ^c (0.08)	68.88 ^a (0.65)	50.72 ^a (1.19)	61.03 ^a (1.24)	0.83 ^b (0.03)

Values are means with standard errors (SE) in parentheses. Different superscript letters within each column represent significant differences ($n=3$, $P<0.05$) among decomposing substrates. AUR denotes acid-unhydrolyzable residue and AHR acid-hydrolyzable residue

Fig. 1 Decomposition responses of needles (a), lower-order roots (b), and higher-order roots (c) of *P. Elliottii* to exogenous N addition. Repeated-measures of ANOVA were used with time as a factor. Significant differences between means were compared using Tukey's test. Data are expressed as means±standard error ($n=3$, $P<0.05$). Open symbols represent control, grey-filled symbols represent low dose of N addition ($40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), and black-filled symbols represent high dose of N addition ($120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$)

concentration in lower-order roots by 9.7 % on average ($P<0.001$, Fig. 2b), but slightly increased those in needles and higher-order roots (Fig. 2a and c). However, the slopes of the AUR concentration increase for higher-order roots (0.08 %) and needles (0.08 %) were higher than that for lower-order roots (0.02 %) ($P=0.002$, Fig. 2a, b, and c). The N concentration in needles varied over the course of decomposition ($P<0.001$, Fig. 2d), with N release at the earlier stage and N immobilization at the later stage (Fig. 2d). For lower-order roots, N release patterns varied among treatments ($P=0.037$, Fig. 2e). Specifically, N immobilization occurred under a high N dose, but N release

occurred under ambient conditions and a low N dose (Fig. 2e). Both low- and high-doses of N stimulated the N immobilization of higher-order roots ($P=0.017$, Fig. 2f). The net AUR remaining was significantly correlated to the net N remaining in lower-order roots ($r^2=0.463$, $P<0.0001$, Fig. 3b), but the relationships were not significant for needles (Fig. 3a) and higher-order roots (Fig. 3c).

Extracellular enzyme activities

Exogenous N addition exerted contrasting effects on microbial EEA, depending on the type of decomposing

Table 2 Decay rate constants (k) and the associated r^2 from negative exponential regression equations of needles and branch order-based roots. Different superscript lowercase letters in each row indicate significant differences of k -values among treatments ($n=3$, $P<0.05$)

Substrate type	Control		Low-N addition		High-N addition	
	k -value	r^2	k -value	r^2	k -value	r^2
Needles	0.246 (0.004) ^a	0.864	0.226 (0.005) ^{ab}	0.891	0.216 (0.006) ^b	0.916
Lower-order roots	0.214 (0.003) ^a	0.810	0.192 (0.009) ^{ab}	0.759	0.182 (0.006) ^b	0.881
Higher-order roots	0.280 (0.009) ^a	0.784	0.273 (0.006) ^a	0.686	0.273 (0.007) ^a	0.749

substrate and enzyme as well as on the dose of N added (Fig. 4a, b, and c). In needles, low- and high-doses of N significantly reduced the activity of NAG by 53.7 % on average ($P=0.001$, Fig. 4a). Adding N at the high rate reduced the activity of BG by 70.6 % ($P=0.006$,

Fig. 4a), of CBH by 87.0 % ($P=0.005$, Fig. 4a), and of PPO by 40.2 % ($P=0.011$, Fig. 4a), whereas N addition had no effect on AP ($P=0.111$, Fig. 4a). In lower-order roots, N addition had no effect on any of the enzyme activities ($P>0.05$, Fig. 4b). In higher-order

Fig. 2 Effects of N addition on AUR remaining (of initial) and N remaining (of initial) in needles (a and d, respectively), lower-order roots (b and e, respectively), and higher-order roots (c and f, respectively) of *P. elliotii* during the course of decomposition. Repeated-measures of ANOVA were used with time as a factor. Significant differences between means were compared using

Tukey's test. Data are expressed as means $\pm$ standard error ($n=3$, $P<0.05$). Open symbols represent control, grey-filled symbols represent low dose of N addition ($40 \text{ kg N ha}^{-1} \text{ yr}^{-1}$), and black-filled symbols represent high dose of N addition ($120 \text{ kg N ha}^{-1} \text{ yr}^{-1}$)

Fig. 3 Mass-based percentage relationships between net AUR remaining (of initial) and net N remaining (of initial) in needles (a), lower-order roots (b), and higher-order roots (c) of *P. Elliottii*. For each type of decomposing substrate, the “net remaining” was

calculated by deducting the AUR (or N) remaining of the control from those of N treatments (the net values at all sampling dates were used, $n=30$, $P<0.05$) to assess the effects of exogenous, but not native N ions in soil on increased AUR concentration

roots, low- and high-doses of N increased the activity of BG by 73.1 % ($P=0.007$, Fig. 4c) and of CBH by 194.9 % ($P=0.005$, Fig. 4c) on average. The low dose

of N increased activity of AP by 48.6 % ($P=0.043$, Fig. 4c), whereas N addition had no effect on activity of NAG and PPO ($P>0.05$, Fig. 4c).

Fig. 4 Effects of N addition on microbial EEA (BG, NAG, CBH, AP, and PPO) in needles (a), lower-order roots (b), and higher-order roots (c) among different treatments. One-way ANOVA was used and significant differences between means were compared using Tukey’s test. For each type of enzyme of each decomposing substrate, different lowercase letters indicate significant differences among treatments and “NS” indicates not significant. Data are expressed as means±standard error ($n=3$, $P<0.05$)

Relationships between decay constants (k) and microbial EEA and AUR concentrations

For needles, the decay rate constant (k) was positively and significantly correlated to microbial EEA (r^2 ranging from 0.461 to 0.675, $P < 0.05$, Table 3) except for BG ($r^2 = 0.379$, $P = 0.078$, Table 3), but it was not significantly correlated to AUR ($P = 0.126$, Table 3). Conversely, for lower-order roots, the k was negatively and significantly correlated to AUR ($r^2 = 0.702$, $P = 0.005$, Table 3), but was not significantly correlated to microbial EEA ($P > 0.05$, Table 3). The value of k was not significantly correlated to microbial EEA and AUR for higher-order roots ($P > 0.05$, Table 3).

Discussion

Retarding-effects of N addition on needle and lower-order root decomposition

Exogenous N addition retarded decomposition of both needles and lower-order roots (Fig. 1a and b). However, the underlying mechanisms could be different between the needles and lower-order roots. Previous studies have concluded that N deposition may inhibit decomposition by suppressing microbial enzyme activities (Carreiro et al. 2000) and/or by binding AUR in substrates to inorganic N ions (Fog 1988; Berg and Matzner 1997). We observed that the AUR concentration in lower-order roots increased with N addition (Fig. 2b), but the AUR concentration in needles did not alter with added N (Fig. 2a). Conversely, the activities of cellulose-degrading enzymes (i.e., BG

and CBH), chitin-degrading enzyme (i.e., NAG), and lignin-degrading enzyme (i.e., PPO) in needles were suppressed by N addition (Fig. 4a), whereas the EEA in lower-order roots did not change significantly with exogenous N addition (Fig. 4b). These contrasting response patterns may be explained by examining the differences in substrate quality and the decomposition environment (especially microbial EEA) between the needles and lower-order roots.

Compared with the needles, the lower-order roots decomposed within the soil and contained a higher concentration of AUR, which is indicative of lignin and phenolic substances (Table 1). Thus, the lignin remains and soluble phenolics in lower-order roots may have adsorbed free N ions more easily from the soil, forming highly polymerized and recalcitrant products (new AUR) (Berg and Matzner 1997). The fact that the net AUR remaining was positively correlated to the net N remaining in lower-order roots (Fig. 3b), indicates that exogenous N addition stimulated the binding of AUR to inorganic N ions. Moreover, the decay rate constant (k) of the lower-order roots was negatively correlated to the AUR concentration (Table 3), but was not correlated to microbial EEA, indicating that the suppression of lower-order root decomposition by N addition could be explained by increases in AUR binding to inorganic N ions. However, in contrast to the traditional recognition that the chemical binding reaction generally occurs at the later stage of decomposition (lignin-dominated phase) (Berg and McClaugherty 2014), the AUR concentration in lower-order roots increased promptly on the 69th day of decomposition, but thereafter more slowly with a lower slope (Fig. 2b). This may imply that lower-order roots have a shorter early-

Table 3 Relationships between decay rate constants (k) and microbial EEA and AUR remaining (the mean values during the course of 524 days of decomposition) for needles and branch

order-based roots. All treatments were pooled ($n=9$, $P < 0.05$). “+” represents positive effects, “-” represents negative effects, and “n.” represents neutral effects

Parameter	Needles			Lower-order roots			Higher-order roots		
	Effects	r^2	P -value	Effects	r^2	P -value	Effects	r^2	P -value
AUR	-	0.301	0.126	-	0.702	0.005	n.	0.036	0.621
BG	+	0.379	0.078	n.	0.038	0.616	n.	0.028	0.667
NAG	+	0.461	0.044	n.	0.150	0.304	n.	0.100	0.406
CBH	+	0.627	0.011	n.	0.016	0.744	n.	0.013	0.771
AP	+	0.526	0.027	n.	0.144	0.314	n.	0.003	0.892
PPO	+	0.675	0.007	n.	0.418	0.060	n.	0.041	0.603

stage of decomposition (carbohydrate-dominated phase) (Fan and Guo 2010) and that an upper limit holds for the binding reaction of AUR to N ions in decomposing tissues (Berg and McClaugherty 2014).

In contrast, the reduced decomposition of needles following N addition could, to a large extent, be ascribed to the reduced microbial EEA (Fig. 4a). Needles were generally decomposed on the soil surface where the microenvironment of microbial decomposers may be more easily modified by a changing environment, such as high N loading (Matulich and Martiny 2014). Microbial EEA has been regarded as the proximate agent of organic matter decomposition (Burns et al. 2013). According to the “stoichiometric decomposition hypothesis”, exogenous nutrient and energy supplies are aligned with increased EEA production to maintain the stoichiometric homeostasis (Hessen et al. 2004). On the contrary, the high dose of N had inhibitory effects on microbial EEA. This may be alternatively explained by the “microbial N mining hypothesis” that high exogenous N supply would alleviate microbial demand for N, and thus reduce utilization of labile C and the decomposition of recalcitrant C (Moorhead and Sinsabaugh 2006; Craine et al. 2007). Furthermore, the decay rate constant (k) of needles was positively correlated to microbial EEA, but not correlated to AUR concentration (Table 3), indicating that the reduced microbial EEA contributed greatly to the suppressed decomposition of needles.

Contrasting responses of lower- and higher-order root decomposition to N addition

Order-based roots within fine root systems presented varying decomposition responses to N addition (Fig. 1b and c). This finding indicates that studying N effects on decomposition of fine roots solely based on diameter-size class may mask the finer response patterns and/or bias the prediction of fine root decomposition response to N addition. Among the branching fine root systems, lower-order roots (absorptive fine roots) are mostly responsible for mutualistic symbioses between plant roots and soil mycorrhizal fungi (Guo et al. 2008) and usually produce a range of defensive secondary compounds (e.g., chitin, tannin, and cutin) to avoid feeding by root herbivores (Sun et al. 2011). Thus, lower-order roots generally possessed high AUR concentration compared with higher-order roots (Xiong et al. 2013). We found that N addition remarkably increased the AUR concentration in lower-order roots, but not in higher-

order roots. Hence, the differential decomposition responses to N addition observed in lower- and higher-order roots may also be explained by the different degrees of binding of AUR to inorganic N ions.

In contrast to our second hypothesis, N addition exerted no effect on decomposition of higher-order roots (Fig. 1c). It has been suggested that microorganisms preferentially utilize root litters compared to foliage litters (Guo et al. 2013) and utilize high-quality compared to poor-quality substrates (Berg and McClaugherty 2014). Given the retarded decomposition of needles and lower-order roots, we expected the decomposition of higher-order roots to be stimulated to meet the needs of microbial decomposers for C sources. However, although levels of microbial EEA (BG, CBH, and AP) in higher-order roots were stimulated by N addition (Fig. 4c), the expected increase in decomposition rate did not occur, and we found no significant relationships between the decay constant (k) and microbial EEA (Table 3). This agrees with a previous study that shows no correspondence between the effects of N on microbial EEA and decomposition (Keeler et al. 2009). It has been surmised that the binding reaction of AUR to N ions may be a potential factor that could offset the increased EEA (Ågren et al. 2001). However, the AUR concentration in higher-order roots was only slightly increased by N addition (Fig. 2c), which may to a limited degree explain the uncoupling of microbial EEA and decomposition of higher-order roots. In light of the temporal heterogeneity of EEA and enzyme diversity involved in decomposition, we could not rule out the possibility that the limited species of enzyme and number of EEA measurement times may be responsible for not successfully capturing the potential intimate relationship between EEA and decomposition (Keeler et al. 2009) in higher-order roots. Thus, more targeted studies and efforts are needed to uncover reasons for the perplexed paradox.

N-dose effects on needle and order-based root decomposition

As predicted, different doses of N addition exerted varying effects on decomposition of lower-order roots (Fig. 1b, Table 2). This finding concurs with previous research that the effects of N addition on root decomposition are dose-specific (Mao et al. 2011). The high dose of N exerted a stronger retarding effect on the decomposition of lower-order roots that could be explained by

its stronger stimulative effect on the binding of AUR to N ions. It has been reported that the binding reaction of AUR to N ions under field conditions could be stimulated by an increase in ammonium levels (Axelsson and Berg 1988). We found that the AUR concentration in lower-order roots was significantly increased by a high-dose ($P < 0.001$, Fig. 2b), but not a low-dose of N, which may be responsible for the differential decomposition responses to N dose. Moreover, the intimate relations between net AUR remaining and net N remaining in lower-order roots ($r^2 = 0.463$, $P < 0.0001$, Fig. 3b) may also confirm the empirical relationships that a good supply of N may give a higher AUR concentration (Berg et al. 2013; Berg and McLaugherty 2014).

In parallel, the decomposition of needles was significantly inhibited by a high dose of N ($P = 0.027$, Fig. 1a; Table 2), but unaffected by a low dose of N. The “N-saturated hypothesis” and “stoichiometric ratio theory” jointly imply that microorganisms have a given demand for N (Aber et al. 1989; Cleveland and Liptzin 2007). The contrasting effects of N addition indicated that the decomposition of needles would be inhibited as the dose of N added exceeds a threshold at which microbial growth and activities would be suppressed. Microbial decomposers are capable of adapting to a changing environment through reshaping community structure (Strickland et al. 2009; Wallenstein and Hall 2012) and/or altering EEA (Carreiro et al. 2000; Saiya-Cork et al. 2002; Keeler et al. 2009). Our results showed that the activities of cellulose-degrading (i.e., BG and CBH) and lignin-degrading enzymes (i.e., PPO) were decreased under a high N dose but not a low N dose (Fig. 4a), which may help explain the differential effects on needle decomposition. An alternative explanation for the lack of any response to a low dose of N may be that the inorganic N in the acidic soils, in this region, is generally dominated by NH_4^+ (Zhang et al. 2013), thus microorganisms may have evolved a range of strategies to adapt themselves to the NH_4^+ -dominant soil environment. As such, low doses of NH_4^+ input may exert little influence on decomposition if such doses do not exceed the tolerance range of microbes.

Conclusions

In general, exogenous N addition exerted detrimental effects on the decay of three types of decomposing materials (negative effects on both needles and lower-

order roots, and neutral effects on higher-order roots) of *P. elliotii*. Given the elevated N deposition in tropical and subtropical forests (Matson et al. 1999), the decomposition of rapid-turnover organs of plants may be inhibited via reducing microbial EEA and/or increasing the binding reaction of AUR to inorganic N ions, which may provide critical mechanisms of C sequestration and N retention in the soil.

Our findings, together with previous studies indicate that the decomposition of dead plant organs may fail to mirror each other at ambient conditions (Hobbie et al. 2010) or in response to N deposition (Norris et al. 2013), in terms of the decay rate (e.g., needles versus lower-order roots) and response direction (e.g., needles versus higher-order roots). Furthermore, the contrasting effects of N addition on lower- and higher-order roots indicate that considering the tissue chemistry heterogeneity within fine root systems may facilitate a more accurate prediction of root decomposition responses to N loading and may help illuminate the relationship between fine root decomposition and N availability.

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