

Do Root Modules Still Exist after they Die?

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Abstract

Background: The terminal branch orders of plant root systems are increasingly known as an ephemeral module. This concept is very crucial to recognize belowground processes. However, it is unknown if root modules still exist after they die?

Methods: The decomposition patterns of the first five root orders were observed for 3 years using a branch-order classification, a litter-bag method and sequential sampling in a common subalpine tree species (*Picea asperata*) of southwestern China.

Results: Two root modules were observed during the 3-year incubation. Among the first five branch orders, the first three order roots exhibited temporal patterns of mass loss, nutrients and stoichiometry distinct from their woody mother roots throughout the experimental period. This study, for the first time, reported the decomposition pattern of each individual root order and found a similar decomposition dynamic among ephemeral root branches in a forest tree species.

Conclusions: Results from this study clearly suggest that root modules may also exist after death, while more data are needed for confirmation. The findings may further advance our understanding of architecture-associated functional heterogeneity in fine-root system and also improve our ability to predict belowground processes.

Background

Linking root structures with their functions is very crucial for understanding belowground biogeochemical processes in terrestrial ecosystems (Hishi, 2007; McCormack et al. 2015). For several decades, ephemeral roots have generally been treated as a homogeneous mass compartment, and defined conveniently as all roots less than a certain diameter (commonly 2 mm) (Jackson et al., 1997; Strand et al., 2008). However, even fine roots (< 2 mm in diameter) can often consist of numerous hierarchies (root orders) (Pregitzer et al. 2002; Guo et al. 2008a). It is increasingly accepted that root traits are functionally linked more to root branching order than to root diameter (Goebel et al., 2011).

Current evidence has indicated that distal branch orders are a relatively specific subgroup with characteristic traits, e.g. regarding morphology, physiology, anatomy and lifespan, compared to their higher-order mother roots (Guo et al. 2008b; Liu et al. 2016). Xia et al. (2010) had proposed a new approach with root modules separated into groups with different functions instead of traditional classifications based on root diameter. They termed distal nonwoody lateral branches as ephemeral root modules and tested it in a temperate tree species of northeast China. A recent study has also observed similar patterns in a desert ecosystem (Liu et al. 2016). Although only two studies have so far tested the modular organization of live root system, similar relationships between anatomy and branching architecture were observed in diverse woody species (e.g. Guo et al. 2008b; Valenzuela-Estrada et al. 2008). Consistent with this deduction, it is possible that the structural and functional modular

organization of live root system is likely to be widespread in terrestrial ecosystems. However, the fate of the different root modules still remains unclear after they die.

Despite the importance of root architecture and decomposition, little attention has been paid to root decomposition of different branches because collecting root segments of distal branch orders is commonly very labor-intensive and time-consuming (Kong and Ma 2014). Global analyses have indicated that fine root decomposition is strongly associated with root tissue chemistry, especially root nitrogen concentration and ratio of carbon to nitrogen (Silver and Miya 2001; Zhang and Wang 2015). Moreover, several studies have found that mycorrhizal fungi may determine the quality of root litter, and further regulate its decomposition rates in some forest tree species (Langley and Hungate 2003; Goebel et al. 2011). It has been widely proved that lateral branches as a whole exhibited distinct nutritional and mycorrhizal patterns from their higher-order mother roots throughout their life history (e.g. Guo et al. 2008b; Liu et al. 2016). In this study, the specific question addressed in this study is that does root modular organization affect after-life processes? Our hypothesis was that the ephemeral roots could have a similar temporal pattern of mass loss, nutrients and stoichiometry distinct from their woody mother roots during the experimental period. Our main objective was to test this hypothesis by exploring the decomposition pattern of each individual branch of the first five root orders in a common subalpine forest tree species (*Picea asperata*) of southwest China.

Methods

Study site

This study was conducted at the Long-term Research Station of Forest Ecosystems of the Sichuan Agricultural University, which is located on the eastern Tibetan Plateau, China. The annual mean precipitation and temperature are 850 mm and 3.0 °C, respectively. In general, seasonal snow cover begins to accumulate in November and melts in March of the following year. *P. asperata* was chosen in this study because it is widely distributed in the area. For this study, a *P. asperata* forest stand was used for a root litter incubation experiment. The basic physicochemical properties of soil (0–15 cm) are as follows: organic carbon 85.7 g kg⁻¹, nitrogen 5.7 g kg⁻¹, pH 6.2 and bulk density 1.2 g cm⁻³, respectively.

Root collection and branch order classification

In June 2013, root segments of *P. asperata* were sampled from a *P. asperata* forest (102.56° E, 31.18° N, 3037 m a.s.l.) according to the methods of Xiong et al. (2013). Briefly, root branching samples from the upper soil profile (20 cm) were excavated and then carefully separated lateral root branches from the soil, ensuring the distal root branching orders were still intact. Following the methods of Pregitzer et al. (2002), i.e., the distal roots were designated as the first order; the root from which two first-order roots branched

was classified as the second order, and so on. In this study, the first five branching root orders were identified, and the adhering soil particles were gently brushed and removed.

Litterbag incubation, harvest, and analysis

Air-dried root branches of 1.0000 ± 0.0010 g of each order class were placed in litterbags (5 cm · 5 cm, mesh size 120 μ m). Four plots (5 m · 5 m) were randomly set up in the original forest. For each root order, duplicate sets of litterbags were placed horizontally at a soil depth of 10 cm in each plot late in the growing season (mid October) 2013. One litterbag of each branch order were randomly harvested from each plot on five occasions: early spring 2014 (mid April), late autumn 2014 (mid October), early spring 2015, late autumn 2015, and early spring 2016. In the laboratory, soil particles and other extraneous materials were removed. Cleaned roots were oven-dried at 65 °C to constant mass and weighed.

Root diameter of different branch orders was measured using WinRHIZO image analysis software (Regent instruments, Quebec, QC, Canada). For each sampling date, specific litters of the same branch order were pooled for chemical analyses after determination of the dry mass. The concentrations of total carbon (C) in the root samples was determined using the dichromate oxidation-ferrous sulfate titration method and the total nitrogen (N) and phosphorus (P) were measured using the methods of Kjeldahl and phosphomolybdenum yellow spectrophotometry after digestion with hydrogen peroxide and sulfuric acid, respectively.

Data analysis

Decomposition rates were calculated from dry mass remaining using a single negative exponential decay model: $X_t/X_0 = e^{-kt}$, where X_t/X_0 is the fraction of mass remaining at time t , t the time elapsed in years and k the annual decay constant (Olson, 1963). In this study, all variables of two bags within each plot were averaged, and the plot was considered to be the experimental replicate. One-way ANOVA with Fisher's LSD test was used to identify significant differences among root orders in initial root traits and constant k value. Repeated measures ANOVA was used to examine the effects of root order, sampling date and their interactions on mass remaining, N concentration, P concentration, C/N and C/P. Differences among means were considered significant at the $P < 0.05$ level. All statistical tests were performed using the Software Statistical Package for the Social Sciences (SPSS) version 16.0.

Results

Initial root chemistry

The mean root diameter increased significantly with root branch order, from 0.29 ± 0.02 mm in first-order roots to 0.64 ± 0.07 mm in fifth-order roots (Table 1). However, both N and P concentrations decreased significantly as the root order increased (Table 1). Both N and P contents in first-order roots were approximately 2 times higher than those in fifth-order roots. Conversely, the C/N and C/P ratios increased with increasing root branch order (Table 1).

Table 1
Initial root traits of different branch orders in *P. asperata*

Root order	Root diameter (mm)	N (g/kg)	P (g/kg)	C/N	C/P
order 1	0.29 ± 0.02c	11.1 ± 0.4a	0.81 ± 0.01a	30 ± 2d	410 ± 21d
order 2	0.33 ± 0.03c	9.0 ± 0.1b	0.64 ± 0.01b	39 ± 4c	553 ± 61c
order 3	0.46 ± 0.04b	7.7 ± 0.2c	0.52 ± 0.04c	55 ± 7b	815 ± 92b
order 4	0.50 ± 0.07b	5.7 ± 0.1d	0.49 ± 0.03c	81 ± 5a	945 ± 64b
order 5	0.64 ± 0.07a	5.5 ± 0.2d	0.37 ± 0.03d	89 ± 5a	1322 ± 94a
Different lowercases indicate a significant difference in variables among orders.					

Mass loss and k value

The first five order roots of *P. asperata* had a loss of 15%-32% of their initial mass at the end of incubation (Fig. 1a). Statistical analysis showed that both order and season alone had significant effects on root mass remaining over the experimental period (Fig. 1a). In general, root mass remaining tended to decline with root branch order, following a pattern of the fifth order < the fourth order < the first three orders (Fig. 1a). However, similar patterns of mass remaining were observed among the first three orders during the experimental period (Fig. 1a). The remaining mass exhibited exponential decreasing trends along with the decomposition time (Fig. 1b, R^2 ranged from 0.88 to 0.93, all $p < 0.001$). The values of decay constant (k) did not differ among the first three orders, which, however, were significantly lower than those of the fourth and fifth order roots (Fig. 1b).

N and P concentrations

Generally, the N concentrations of the first three order roots increased significantly during the first non-growing season and then began to decrease gradually (Fig. 2a). There were significant differences in initial N concentration across the first three order roots but the variations among them decreased and even vanished along with the decomposition advancement (Fig. 2a). N concentration was almost identical between fourth and fifth orders over the experimental period. In addition, the seasonal dynamic of N concentration in fourth and fifth orders was relatively small as compared to the distal branch orders (Fig. 2a). Root order, season and their interaction all showed significant effects on root N concentration (Fig. 2a).

The P concentrations of all order roots followed a similar seasonal pattern over the experimental period, with increasing in the non-growing season but decreasing in the growing season (Fig. 2b). The variations in P concentration among the first three orders were much greater during the first year incubation as compared to the later stage of decomposition (Fig. 2b). Similar to N concentration, P concentrations were similar in both fourth and fifth orders over the experimental period, which were generally lower than those

of the first three root orders (Fig. 2b). Statistical analysis indicated that order, season and their interaction all exhibited significant influences on P concentration (Fig. 2b).

Stoichiometric ratios

Contrary to N concentration, the C/N ratios significantly decreased over the first year of decomposition then tended to increase gradually (Fig. 3a). The C/N ratio increased with increasing root branch orders and this pattern almost existed throughout the whole experiment (Fig. 3a). The C/N ratios of the first three order roots were significantly lower than those of the last two orders (Fig. 3a). The seasonality of C/N ratio was similar among the first three orders, which was different from those of higher orders (Fig. 3a). ANOVA analysis showed that the effects of order, season and their interaction were significant on C/N ratio (Fig. 3a).

The C/P ratios of all orders showed a similar seasonal dynamic during the 3-year decomposition, with decreasing in the non-growing season but increasing in the growing season (Fig. 3b). The variation of C/P ratio in the early stage of decomposition was stronger relative to other stages (Fig. 3b). Except for the initial value, the C/P ratio was similar between fourth and fifth orders during the experimental period, which was often greater than those of the first three orders (Fig. 3b). Likewise, the effect of root order on C/P ratio was strongly dependent on seasons (Fig. 3b).

Discussion

Root decomposition is a critical process in global carbon cycling in terrestrial ecosystems, as it may influence ecosystem productivity via regulating nutrients cycle (Hobbie, 1992). While most of the prior studies on decomposition have mainly focused on aboveground litter, recent evidence suggest that root-derived carbon is retained more efficiently in soils and microorganisms than that of aboveground plant litter (Kramer et al. 2010; Mendez-Millan et al. 2010). It is increasingly recognized that ephemeral root modules (the most dynamic part) in a root branch is closely associated with a number of ecological processes as a result of its fast turnover rate, high nutrient content and strong physiological activity (Xia et al. 2010; Long et al. 2013; Liu et al. 2016). Moreover, given the importance of distal root branch orders in carbon and nutrient cycling during the decomposition process, it gained a great interest in unraveling the decomposition patterns of ephemeral root modules (McCormack et al. 2015; Lin and Zeng 2017). However, the pattern of individual order of ephemeral root modules remains unclear after they die.

The main objective of this study was to test whether the behavior associated with root modules within the fine root branching system would translate into the performance during the decomposition process. In this study, the temporal decomposition pattern of each individual order of the first five branches was investigated over the 3-year field incubation using a litter-bag method in a common subalpine tree species (*P. asperata*) of southwestern China. Our data support the hypothesis that root modules did also exist in the decomposition process, at least in *P. asperata* we studied. We could clearly distinguish two

types of root module in mass remaining, nutrient concentration and stoichiometry during the 3-year experiment.

First of all, in *P. asperata* we investigated, lateral branches composed of the first three orders have a significantly slower decomposition rate than their woody mother roots. The mass remaining was always greater in the distal root branch orders than in their mother roots, especially after first-winter decomposition. Over the 915-day field incubation experiment, only 15–17% of the distal root branch orders decomposed, in contrast to the mass loss rate of 30–32% in their woody mother roots. In addition, this finding was also supported by the decay constant (k) based on an exponential model (3-year experimental data). The values of decay constant (k) were similar among the first three orders, which, however, were much lower than those of their woody mother roots (fourth- and fifth-order roots). Thus, the distal root branch orders (the first three orders) and higher-order roots (fourth- and fifth-order roots) may form two contrasting groups within regard to mass loss, suggesting that there was a similar functional trait (i.e., decomposition) within each module group.

Moreover, the distal root branch orders as a whole exhibited distinct nutritional pattern throughout the 3-year decomposition experiment. The N and P concentrations between fourth and fifth orders were almost identical over the experimental period, which were significantly lower, however, than those of the distal root branch orders. Additionally, the first three root orders, which had higher N and P concentrations, exhibited marked seasonal patterns in the 3-year decomposition process. On the contrary, the temporal pattern of N and P concentrations was relatively slight in the higher-order roots (fourth- and fifth-order roots) during the experimental period. Therefore, the distal root branch orders and their mother branches formed two distinct subgroups in N and P concentration throughout the 3-year experimental period, implying that a similar function (i.e., nutrient cycling) could exist within each module group. Previous studies were unable to recognize this phenomenon well, mainly because the root samples used for decomposition experiment are differentiated by diameters (e.g. Sun et al. 2013; Wang et al. 2014) or combined orders (e.g., order 1 + order 2, Fan and Guo 2010; Goebel et al. 2011).

Lastly, two root module organizations were also observed in the litter stoichiometry of C and nutrients along with the decomposition advancement. The values of C/N and C/P in both fourth and fifth orders were similar over the experimental period, which generally were much higher than those of the distal root branch orders. Moreover, the distal root branch orders as intact lateral module showed a highly synchronous dynamic in C/N and C/P in the decomposing root litters. A great number of studies have demonstrated that litter quality, particularly the stoichiometry of C and nutrients as well as the chemistry (i.e. N and P concentration), regulate not only rates of litter mass loss, but also the nutrients cycling (i.e., Manzone et al. 2010; Mooshammer et al. 2012). Therefore, the contrasting pattern in nutrients (i.e., N and P concentrations) and stoichiometry of C and nutrients (i.e., C/N and C/P) in the decomposing root litters between the distal root branch orders and their mother branches may, to large extent, represent distinct functional traits of mass loss and microbial nutrient release and immobilization.

At the global scale, the roots with higher nutrient concentrations and lower C:N ratio may decompose faster (Fernandez and Koide 2014; Zhang and Wang 2015). Our study has found that the N and P concentrations decreased but C/N and C/P ratios increased with increasing root branch order in the initial chemistry of *P. asperata* (Table 1). Therefore, the decomposition rate is expected to decrease with root branch orders in *P. asperata*. On the contrary, over a 3-year observation period, the distal branch order roots (the first three orders) decomposed much slower than their mother branches (the fourth and fifth order), as reflected by significant order effects (Fig. 1ab). Several untested underlying mechanisms may be responsible for the observed results. On the one hand, the distal branch order roots are preferentially colonized by fungi and encased by a fungal sheath (Guo et al. 2008b). The fungal sheath is generally fibrous and rich in recalcitrant components (e.g., chitin) (Swift et al. 1979). Therefore, the physically and chemically resistant boundary of fungal sheaths may need much more time for microbes to break down (Langley et al. 2006). *P. asperata* noted in our study is a typical tree species with ectomycorrhizal fungi (EM) (Song et al. 2006). Moreover, a considerable number of fungal sheaths had been observed in the lower order roots of *P. asperata* (unpublished data). Obviously, our results may also, to some degree, support the mycorrhizal hypothesis, while additional data are needed for confirmation. On the other hand, recent experiments also reported that initial root traits, such as carbon quality and quantity (e.g., total nonstructural carbohydrate and acid insoluble fraction), nutrient contents (e.g., N concentration) and recalcitrant components (e.g., tannin), may in part account for the observed slow decomposition of lower-order roots in some given forest tree species (e.g., Fan and Guo 2010; Sun et al. 2013; Fernandez and Koide 2014; Dong et al. 2016), while the underlying mechanisms of specific factors have not been clearly elucidated. Such inconsistent findings imply that diverse mechanisms may regulate the decomposition of fine-root system. Lastly, some root traits often increase or decrease linearly with increasing branch order (e.g., tissue chemistry) but branch-trait relationships are strongly nonlinear in some cases (e.g., mycorrhizal colonization) (McCormack et al. 2015), which reflect complex controls over the decomposition pattern within a fine-root system.

Implications

The findings in this study have the following important implications. Firstly, to our knowledge, few studies have proved that ephemeral live root module generally exists in the distal branches (i.e., the first three branch orders) through investigating the life-span, respiration, anatomy and N concentration of each individual order of fine-root system in some forest tree species (Guo et al. 2008b; Xia et al. 2010; Liu et al. 2016). In recent years, a growing number of studies have examined the heterogeneity in root decomposition among root order groups in some given tree species (see review, Lin and Zeng 2017). In these studies, the first two branch roots were generally combined to a class and the last two (order 3 + order 4) or three (order 3-order 5) branch roots was combined to another one (e.g., Fan and Guo 2010; Goebel et al. 2011; Sun et al. 2013). The segments of distal branch orders (the first two branch roots) seem to have been widely accepted and demonstrated as a functional module organization during the after-life decomposition process. However, no direct available evidence suggests that there are no variations in decomposition rate among the distal branch orders (e.g., the first three branch roots). Our

observations presented in this study clearly indicated that the decomposition dynamic is similar among the first three root branches in *P. asperata* over the 3-year incubation. The results also found that the fine-root system of *P. asperata* can be divided into two modules (the first three orders vs. the last two orders) in the decomposition dynamic. Consistent with this deduction, the distal branch order roots (the first three orders) can be regarded as a relatively independent organization to estimate the belowground root-derived C and nutrients cycling in forest ecosystems dominated by EM tree species, at least in *P. asperata* forest. Additionally, exploring the decomposition patterns of ephemeral root module [the first three orders] could be more accurate and reliable to evaluate the belowground C and nutrient cycling in some specific forest ecosystems.

Secondly, the two contrasting root modules observed in this study may also imply that collecting root segments of the distal branch orders would not be as labor-intensive and time-consuming as before. This is mainly due to the fact that the first three branch orders can be considered to be a similar functional organization after death in forest tree species. A recent study has also demonstrated that hand-kneading approach is a convenient and reliable way to acquire ephemeral root module (Kong & Ma 2014). It is important to note that the live ephemeral root model occurs in the form of the first two root orders in some shrub species from a desert system (Liu et al. 2016). Therefore, merging the distal three root orders is not necessarily to be the ideal module for all species and sites. Obviously, more plant species associated with EM are badly needed to test the generality of fine-root modules after death.

Finally, a great number of studies have shown that lower-order roots generally had high turnover and mortality rates (e.g., Guo et al. 2008a; Xia et al. 2010). However, consistent with the reported results, the mass loss rates of the distal branch order roots was very slow in the 3-year decomposition as a result of diverse untested mechanisms. Thus, these traits in lower-order roots are likely to be favorable to soil C and nutrient retention in the forest ecosystems dominated by EM tree species. It should be mentioned that the mesh size of litterbag used in this study was so fine that efficiently prevents root segment loss from litterbags. However, such small size could, to some extent, underestimate decomposition rate due to the absence of soil fauna, especially large-sized and medium-sized soil animals.

Conclusions

In summary, this study explored the temporal patterns of mass loss, nutrients contents and stoichiometry of C and nutrients of the first five branch order roots in the 3-year decomposition in a common subalpine forest tree species (*P. asperata*) of southwestern China. Two root modules were detected in the 3-year decomposition experiment. Among the first five branch orders, the first three root orders of *P. asperata* showed slower mass loss, higher N and P concentrations and lower C/N and C/P ratios during the experimental period as compared to the last two branch order roots. The nutrients concentrations and stoichiometric ratios within the first three branch orders significantly converged as the decomposition proceeded. Moreover, the temporal pattern of the measured variables was similar within each module group, but was more or less different between the module groups over the experimental period. To our knowledge, this study is the first to report the decomposition patterns of each individual order of the first

five root branches in a forest tree species. Moreover, our data clearly suggested that the decomposition pattern was similar among the ephemeral root branches in *P. asperata*. Here, we provide a piece of evidence that root modules do also still exist, at least in the first 3 years of decomposition in the fine root system of *P. asperata*. Obviously, this study made further attempt for understanding the structure-function linkage after fine-root death. Certainly, additional detailed work to integrate potential factors and explore their relative importance would help us to better understand and predict below-ground root dynamic in forest ecosystems.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

#JHL and CMY contributed equally. ZFX conceived the study. ZFX, HL, BT and LXW provided project support. JHL and SNL performed the research and analyzed the data. ZFX, JHL and CMY wrote the

manuscript. BT and ZFX contributed to editing. All authors contributed to the work and gave final approval for publication.

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Figures

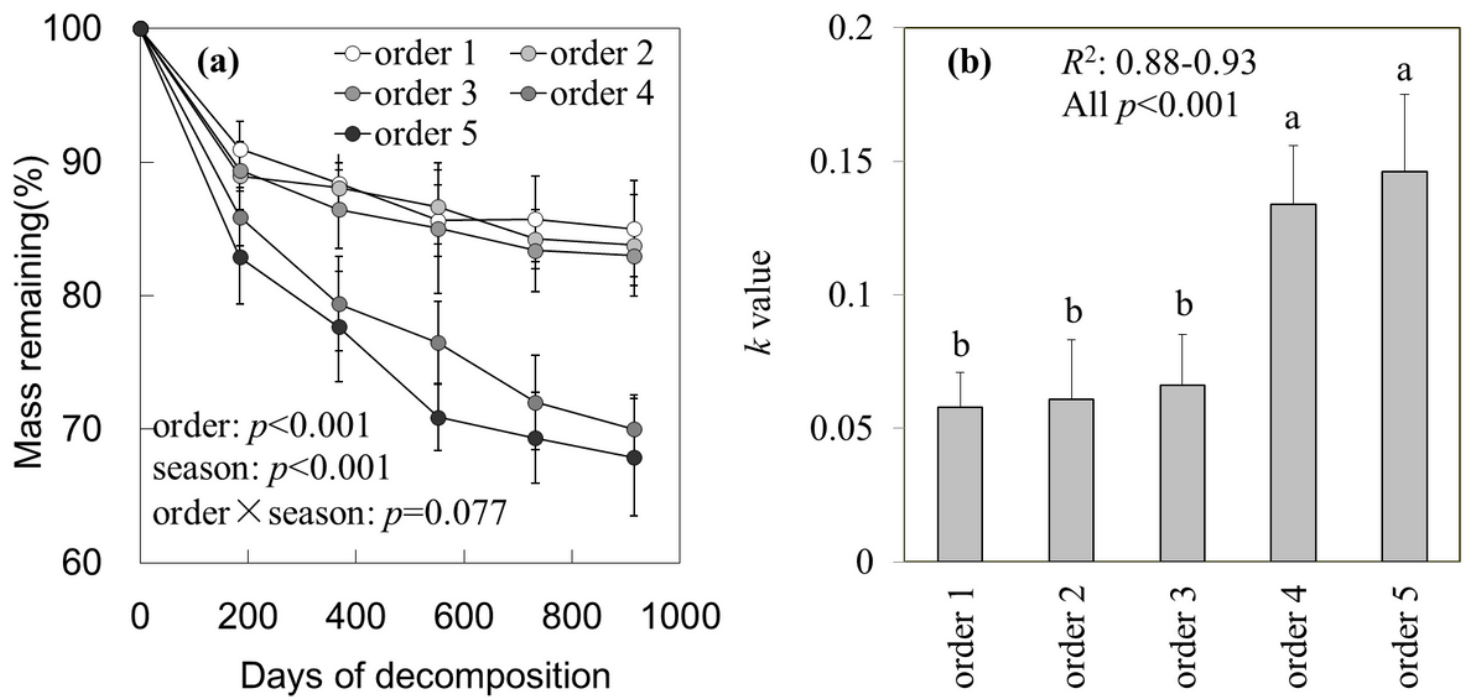


Figure 1

Mass remaining (%) and decay constant (k) of the first five root branch orders in *P. asperata* during the 3-year decomposition. Values are means $\pm$ S.D.

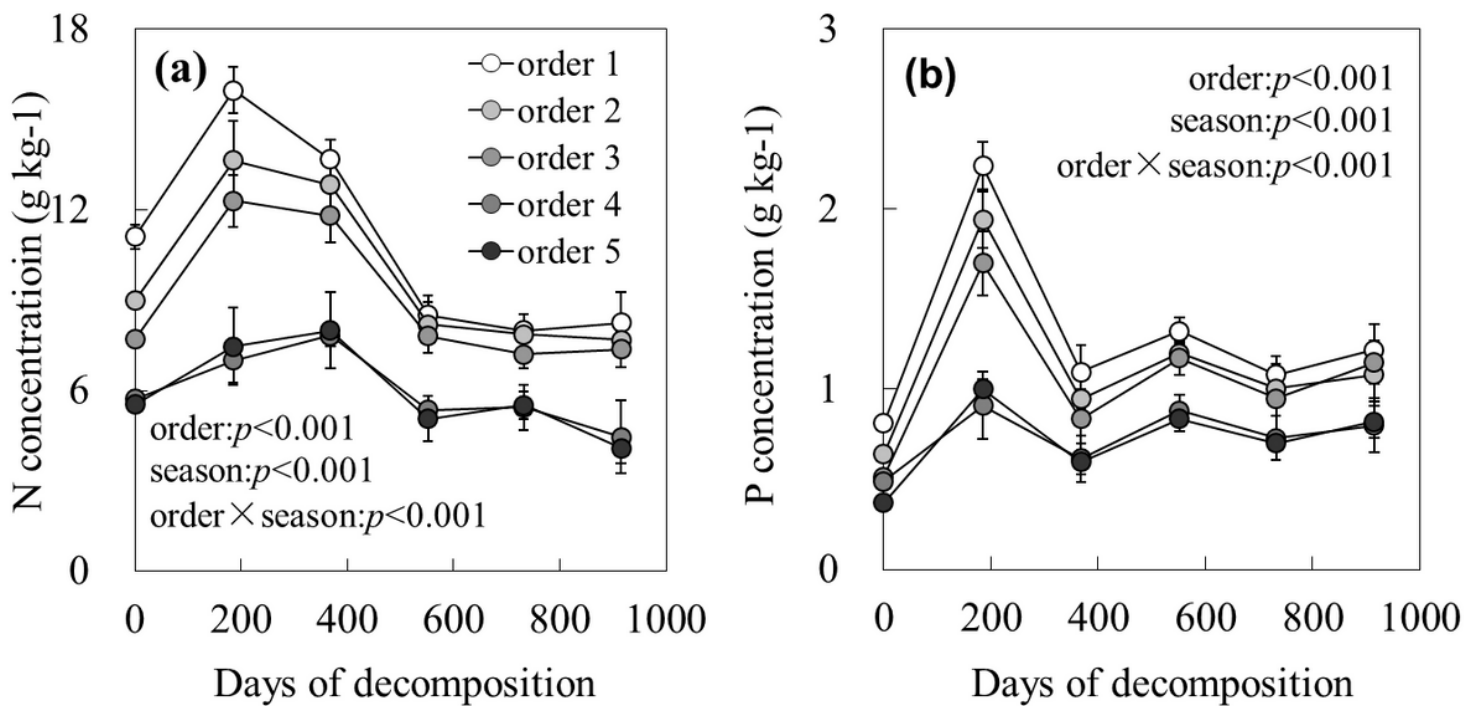


Figure 2

Seasonal patterns in N and P concentrations of the first five root branch orders in *P. asperata* during the 3-year decomposition period. Values are means $\pm$ S.D.

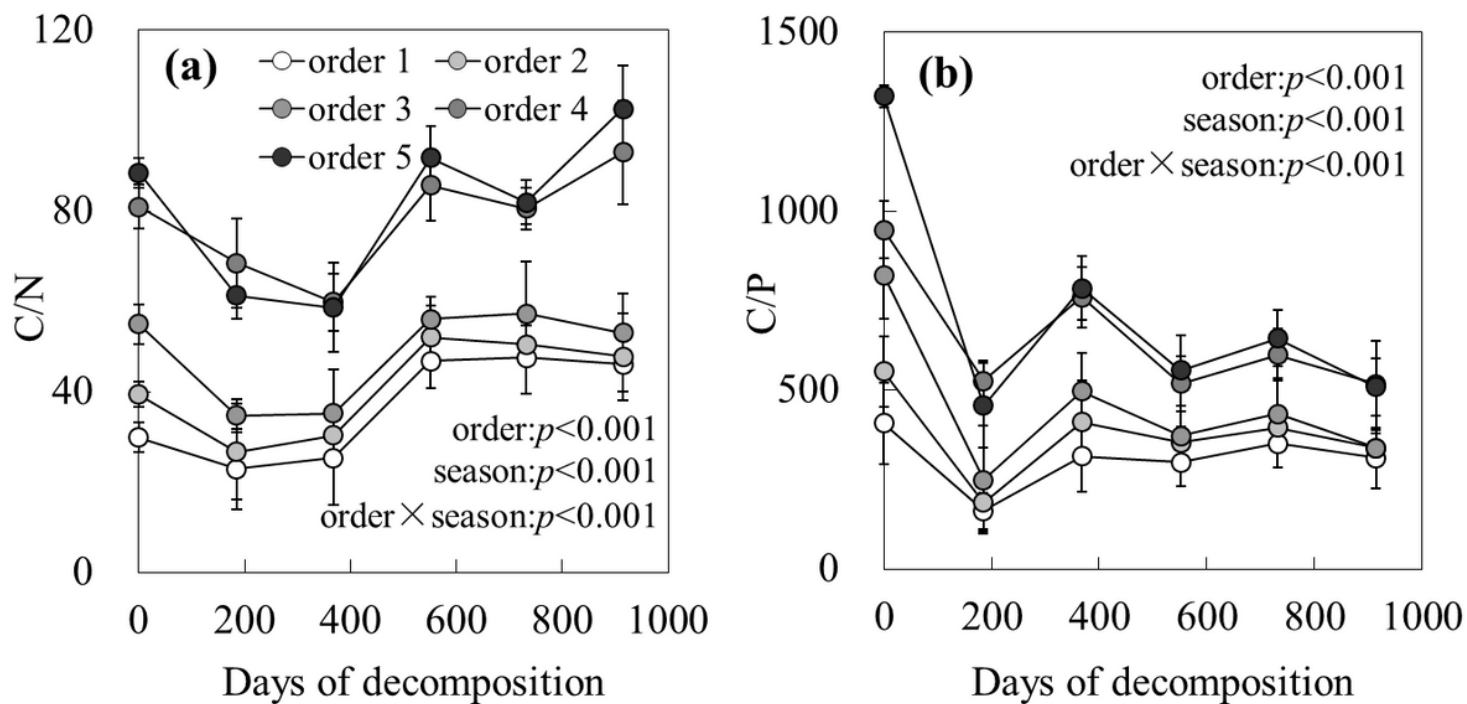


Figure 3

Seasonal patterns in C/N and C/P ratios of the first five root branch orders in *P. asperata* during the 3-year decomposition period. Values are means $\pm$ S.D.