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Decomposition of the finest root branching orders: linking belowground dynamics to fine-root function and structure

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Abstract. Root turnover is fastest in the finest roots of the root system (first root order). Additionally, tissue chemistry varies among even the finest root orders and between white roots and older, pigmented roots. Yet the effects of pigmentation and order on root decomposition have rarely been examined. We separated the first four root orders (all <1 mm) of four temperate tree species into three classes: white first- and second-order roots; pigmented first- and second-order roots; and pigmented third- and fourth-order roots. Roots were enclosed in litterbags and buried under their own and under a common species canopy in a 34-year-old common garden in Poland. When comparing decomposition of different root orders over 36 months, pigmented third- and fourth-order roots with a higher C:N ratio decomposed more rapidly, losing 20–40% of their mass, than pigmented first- and second-order roots, which lost no more than 20%. When comparing decomposition of roots of different levels of pigmentation within the same root order over 14 months, pigmented (older) first- and second-order roots lost ~10% of their mass, while white (younger) first- and second-order roots lost ~30%. In contrast to root mass loss, root N content declined more rapidly in the first- and second-order roots than in third- and fourth-order roots. In higher-order roots, N increased in the first 10 months from ~110% to nearly 150% of initial N content, depending on species; by the end of the study N content had returned to initial levels. These findings suggest that, in plant communities where root mortality is primarily of pigmented first- and second-order roots, microbial decomposition may be slower than estimates derived from bulk fine-root litterbag experiments, which typically contain at least four root orders. Thus, a more mechanistic understanding of root decomposition and its contribution to ecosystem carbon and nutrient dynamics requires a fundamental shift in experimental methods that stratifies root samples for decomposition along more functionally based criteria such as root order and pigmentation, which parallel the markedly different longevities of these different root classes.

Key words: Acer pseudoplatanus L.; biomass; decomposition; fine roots; Larix decidua Mill.; litterbags; nitrogen; Pinus sylvestris L.; root order; root pigmentation; Siemianice Experimental Forest, southwestern Poland; Tilia cordata Mill.

Introduction

While fine-root turnover may represent one-third to one-half of total plant tissue turnover (Caldwell 1987, Jackson et al. 1997), the fate of the finest roots after they die is poorly understood. Previous studies suggest that root decomposition is strongly influenced by tissue chemistry. For example, in a global meta-analysis of data in 32 publications (representing 30 study sites and decomposition of fine roots of over 20 conifer and broadleaf tree species), rates of fine-root decomposition were more closely linked to root tissue chemistry

(specifically root Ca concentration and C:N ratio) than to climatic or other environmental factors (Silver and Miya 2001). Root tissue chemistry explained greater than 85% of the variation in root decay rates, in both the initial and later phases of root decomposition. Important chemical properties linked to fine-root decomposition in temperate tree species include Ca, lignin, and N concentrations; C:N, lignin:N, and lignin:P ratios; and concentrations of water-soluble phenols (Berg 1984, Ostertag and Hobbie 1999, Silver and Miya 2001, Chen et al. 2002).

Variation in tissue chemistry and anatomy is functionally linked more to root branching order than to root diameter (Pregitzer et al. 2002, Guo et al. 2008*a*, Valenzuela-Estrada et al. 2008). Different root orders

perform different sets of functions with higher orders (e.g., third, fourth, and higher) principally used in transport, storage, and structural support and lower orders (e.g., first and second) used mainly for nutrient and water acquisition (Guo et al. 2008a, Valenzuela-Estrada et al. 2008, 2009). These differences in function among root orders strongly influence tissue chemical properties, including C:N ratio, and concentrations of total nonstructural carbohydrates, Ca, phenolic compounds, suberin, and lignin (McKenzie and Peterson 1995a, b, Pregitzer et al. 2002, Guo et al. 2004, Leuschner et al. 2004, Soukup et al. 2004, Fan and Guo 2010). In *Pinus palustris* for example, first-order roots (mean diameter = 0.35 mm) had approximately fourfold higher N concentration than fourth-order roots (mean diameter = 0.79 mm) (control plots; Guo et al. 2004). Moreover, root order also strongly influences life span, with life span increasing by root order such that third- and fourth-order roots more often are semipermanent members of the root system (Eissenstat et al. 2000, Wells and Eissenstat 2001, Guo et al. 2008a, b). Consequently, the first- and second-order roots that most frequently die and that are chemically distinct from the third- and fourth-order roots may decompose at different rates from these higher-order roots; yet typical "fine"-root decomposition studies with a diameter cutoff of less than 1 or 2 mm likely include at least four root orders (Pregitzer et al. 2002, Guo et al. 2004, 2008a).

Rarely have studies of root decomposition accounted for potentially important variation in tissue chemistry among fine-root diameter classes or branching orders within the root system, and even fewer have focused on the finest, lowest-order roots that dominate root turnover. An exception is a study by Fan and Guo (2010) that revealed that despite a higher C:N ratio in higher-order than lower-order roots, the lower-order (orders 1 and 2) roots decomposed more slowly over a 500-d period in two tree species in forest plantations in northeastern China. They also found total nonstructural carbohydrate concentration to be positively correlated with rates of decomposition.

In addition to root order, another important source of variation in root chemistry is pigmentation. During a growing season, flushes of fine-root growth can occur in distinct periods (Engler 1903, Krueger and Trappe 1967, Lyr and Hoffmann 1967, King et al. 2002), resulting in discrete subpopulations of first- and second-order roots that are slightly older and pigmented vs. younger and unpigmented ("white") roots. To our knowledge, roots always begin white and change color on the order of days to months, depending on the species and the environment (Comas et al. 2000, Wells and Eissenstat 2003, Withington et al. 2006). The change in color usually represents changes in tissue chemistry, anatomy, and mycorrhizal colonization (Comas et al. 2000, Wells and Eissenstat 2003). Younger, white first- and second-order roots may have much lower C:N ratio, concentrations of phenolic compounds, and secondary wall

development than pigmented roots that may be only one month older (Eissenstat and Volder 2005, Volder et al. 2005). Young, white roots may also be particularly vulnerable to predation and parasitism because of their limited defenses (Bauerle et al. 2007, Resendes et al. 2008). Thus, roots that die when young and still unpigmented may decompose at different rates than those that die at a later pigmented stage, contributing to variation in rates of carbon and nutrient cycling. While white roots are vulnerable to herbivory, a large fraction of white roots damaged by herbivores may not actually pass through the gut of the organisms and thus follow a more typical path of microbial decomposition (Bauerle et al. 2007, Resendes et al. 2008).

The main objective of this study was to determine the effect of root order and root pigmentation on root tissue chemistry and rates of decomposition among four widespread and important European temperate tree species that vary widely in tissue traits (e.g., root Ca concentrations, mycorrhizal type; Hobbie et al. 2006, Dauer et al. 2007). To address the effects of root order, we contrasted rates of decay among pigmented first- and second-order roots with those of pigmented third- and fourth-order roots. Lower-order roots typically have smaller diameters, higher N and P concentrations, lower lignin concentrations, lower nonstructural carbohydrate concentrations, and higher mycorrhizal colonization levels than do higher-order roots (Peterson et al. 1999, Leuschner et al. 2003, Hishi 2007, Guo et al. 2008a, Valenzuela-Estrada et al. 2008, Fan and Guo 2010). Pigmented third and fourth higher-order roots are more likely to develop secondary vascular tissues, including larger portions of xylem elements with lignified secondary walls, and loss of the cortex and associated mycorrhizas with secondary growth (Esau 1965, Brundrett et al. 1990, Massicotte et al. 1999, Guo et al. 2008a). If decomposition was primarily driven by C:N ratio (as commonly seen more generally; Silver and Miya 2001), than we would expect that first- and second-order roots would decompose faster than third- and fourth-order roots.

We also expected root pigmentation to affect rates of decomposition. Compared to older, pigmented first- and second-order roots, younger, white first- and second-order roots typically have lower concentrations of condensed tannins, less secondary wall development with less lignin and suberin deposition, and lower C:N ratios (Peterson et al. 1999, Comas et al. 2000; Volder et al. 2005). Because of these differences, we hypothesized that white fine roots would decompose more rapidly than roots of similar order that are pigmented.

METHODS

Site description

The experiment was established in the common garden "Morawina" of the Siemianice Experimental Forest near the village of Biadaszki in southwestern Poland (51°14.87′ N, 18°06.35′ E, elevation 150 m). We

examined root decomposition of four temperate tree species of 14 growing at that site: sycamore maple (*Acer pseudoplatanus* L.), small-leafed lime (*Tilia cordata* Mill.), European larch (*Larix decidua* Mill.), and Scots pine (*Pinus sylvestris* L.). The species differ in numerous ways at tissue and plant scales, including their litter Ca content, which has been linked to rates of forest floor turnover (Reich et al. 2005, Hobbie et al. 2006). Each single-species plot of the four tree species was 20 × 20 m and replicated three times. Local weather conditions were influenced by the maritime and continental climate with a long-term average annual precipitation of 591 mm and a long-term mean annual air temperature of 8.2°C (Reich et al. 2005). During the time of our decomposition experiment, mean annual air temperature was 7.5°C, with mean monthly maximum of 23.5°C and a mean monthly minimum of −11°C. Buried data loggers (HOBO 8 K, Onset Computer, Bourne, Massachusetts, USA) recorded soil temperature in the center of one plot of each of the four tree species for the first 20 months of the study. From July 2004 until February 2006, mean soil temperature at a depth of 10 cm was 8.3°C (−1.9°C hourly minimum and 19.9°C hourly maximum). The relatively infertile soil of the common garden varied from a gray-brown podzolic to a brown podzolic soil (Spodosol U.S. Soil Taxonomy), the latter being less fertile (Withington et al. 2003). Soil pH of the O horizon in the plots of the four tree species varied from 4.0 (*Pinus*) to 6.1 (*Tilia*), while soil pH was less variable in the upper mineral horizon (0–20 cm), ranging from 4.0 (*Pinus*) to 5.0 (*Acer*) (Reich et al. 2005). Further information about the soil characteristics of the common garden can be found in Hobbie et al. (2006, 2007).

Plant material

Because the amount of first- and second-order roots that were needed for the decomposition studies (2.2 g dry mass) could not be acquired in these long-term plots without excessive disturbance (more than 50 5-cm-diameter, 20-cm-length cores for at least three of the species would have been required), we used plants from local nurseries for root material. We acquired seedlings of two broadleaf tree species (*Tilia cordata*, *Acer pseudoplatanus*) from one local nursery and two coniferous tree species (*Pinus sylvestris*, *Larix decidua*) from a different local nursery in July 2004. Immediately after removal from the nursery, individual root systems were separated from the two- and three-year-old tree seedlings and soaked in 3-L, water-filled containers to remove bulk soil and to avoid the loss of the finest roots. The three ectomycorrhizal tree species (*T. cordata*, *P. sylvestris*, *L. decidua*) were all colonized by ectomycorrhizal fungi (Appendix A: Table A1). For the arbuscular mycorrhizal, *A. pseudoplatanus* (Withington et al. 2003), mycorrhizas were not assessed. Root orders were identified by the morphometric approach, as described by Fitter (1982) and refined by Pregitzer et al. (2002) and

collected from 4–8 cm long fine-root segments, using stand-alone magnifying glasses and tweezers. We combined pigmented first- and second-order roots into one class and pigmented third- and fourth-order roots (root diameter <1 mm) into a second class from ~15 tree seedlings per species, in order to collect enough root mass for a three-year study. In addition, for *A. pseudoplatanus* and *T. cordata* there were sufficient white fresh first- and second-order roots to form a third class. Thus, we had three fine-root classes: fresh growth (white) first- and second-order roots, [1 + 2 W]; pigmented (brown) first- and second-order roots, [1 + 2 B]; and third- and fourth-order pigmented roots, [3 + 4 B]. All fine roots were oven-dried at 65°C to constant mass. Approximately 100 mg of sample material of each root class were then sealed into their own 5 × 10 cm compartment in one Ankom Forage bag (size 10 × 20 cm, nitrogen-free polyester, mesh size 50 μm, Ankom Technology, Macedon, New York, USA).

For the study of root order, five root litterbags (RLB) were buried in August 2004 in a horizontal orientation at a depth of 8–13 cm in the center of each of the three replicate, monospecific tree species plots of the same species as the nursery seedlings ("own" plots). A different RLB of each tree species plot was recovered after 3, 8, 14, 19, and 36 months (three RLB in total for each harvest). We used the remaining fine-root-order material and buried three additional RLB for each of the four tree species, also replicated three times, under a common-species canopy (*Acer platanoides* L.) ("common" plots). A different RLB of each tree species under the common canopy was recovered after 3, 8, and 14 months, yielding three RLB in total per species for each harvest.

The study of the effects of root pigmentation on root decomposition was limited by availability of root material; therefore we examined decomposition of [1 + 2 W] roots only in their own plots over a time period of 14 months for two species, *A. pseudoplatanus* and *T. cordata*. Root bags were installed in August 2004 in a similar orientation as previously described, harvested at 3, 8, and 14 months, and compared with pigmented roots of the same order.

At each harvest date, root litter bags were again oven-dried at 65°C to constant mass, and root-order segments were collected for weighing. Because root segments were still intact, complete removal was straightforward. A portion (5 mg) of the roots from each of the three recovered root litter bags from the three different plots (*n* = 3) were used to determine C and N concentrations with an elemental analyzer (Fisons EA 1108; Fisons Instruments, Dearborn, Michigan, USA). A second portion (5 mg) was used to determine Mg, Ca, Al, and P concentrations by inductively coupled plasma-atomic emission spectrometry (ICP) after samples were dry-ashed (500°C) and resuspended in a 1 mol/L HCl matrix (Agricultural Analytical Service Laboratory, University Park, Pennsylvania, USA). A third portion (5 mg) was

TABLE 1.   The effects of root order on initial tissue chemistry at the start of the litterbag experiment in four temperate tree species: *Acer pseudoplatanus* L., *Tilia cordata* Mill., *Larix decidua* Mill., and *Pinus sylvestris* L.

Species	Root order	C	N	Lignin	Al	Ca	Mg	P
Acer pseudoplatanus	[1 + 2 B]	488 (0.2)	15.8 (0.4)	227 (8.5)	1.15 (0.1)	6.2 (0.3)	3.1 (0.2)	1.8 (0.1)
	[3 + 4 B]	493 (6.6)	9.9 (0.6)	229 (39)	0.45 (0.0)	7.3 (0.3)	1.6 (0.0)	1.6 (0.1)
Tilia cordata	[1 + 2 B]	490 (2.0)	16.3 (0.5)	189 (54)	1.47 (0.1)	14 (0.7)	2.5 (0.1)	2.0 (0.1)
	[3 + 4 B]	487 (9.2)	8.6 (0.3)	247 (63)	0.51 (0.0)	7.1 (0.9)	1.7 (0.1)	1.1 (0.1)
Larix decidua	[1 + 2 B]	449 (0.3)	18.9 (0.0)	176 (29)	9.44 (0.3)	7.6 (1.1)	1.9 (0.1)	5.2 (0.1)
	[3 + 4 B]	482 (1.3)	11.8 (0.2)	200 (55)	7.21 (0.4)	6.5 (0.2)	1.9 (0.1)	2.6 (0.1)
Pinus sylvestris	[1 + 2 B]	434 (6.9)	18.0 (0.9)	102 (22)	9.93 (0.4)	7.4 (0.5)	1.9 (0.1)	5.5 (0.2)
	[3 + 4 B]	475 (17)	19.1 (0.5)	129 (22)	7.86 (0.2)	9.7 (0.5)	2.5 (0.1)	5.2 (0.1)

Notes: Data are expressed as milligrams per gram (with SE in parentheses; $n = 3$) of root dry mass of two root order classes: pigmented first- and second-order roots [1 + 2 B] and pigmented third- and fourth-order roots [3 + 4 B] of the four temperate tree species. The experiment was established in the common garden "Morawina" of the Siemianice Experimental Forest near the village of Biadaszki in southwestern Poland.

used for lignin determination by a modified acetyl bromide method (Iiyama and Wallis 1988, Brinkmann et al. 2002) after removing esterified phenolics and lipids. Root mass and lignin concentration are reported on an ash-free dry-mass basis.

Since root segments of root branching orders showed limited superficial signs of decay, [1 + 2 B] and [3 + 4 B] roots of the species were investigated for internal decay of the root structures from the root litter bags after 19 and 36 months of decomposition. Root segments of ~1 cm length of the different branching orders were embedded in Paraplast plus (Sigma Chemical, St. Louis, Missouri, USA), after fixation and dehydration, to obtain 10-μm root-order cross sections, using an American Optics microtome (Warner-Lambert Technologies, Buffalo, New York, USA). Cross sections were stained in 0.5% toluidine blue O in 1% sodium tetra borate according to O'Brien et al. (1968). Digital microscope images of root cross sections, embedded in Canadian Balsam, were taken under an Olympus BX61 Epi-Fluorescence Microscope (Olympus America, Center Valley, Pennsylvania, USA) at the Cytometry Facility at the Huck Institutes of the Life Sciences at Pennsylvania State University, University Park, Pennsylvania, USA.

Calculations and statistics

We analyzed the effects of root order and root pigmentation on initial tissue chemistry concentrations and ratios in the four temperate tree species with ANOVA. Percentage of remaining ash-free dry mass relative to the initial dry mass was determined for each

harvest. We calculated the decay constant (k) of each root class across plots by fitting the proportion of mass-remaining data of each root class to a single, negative exponential decay model:

$$X = e^{-kt}$$

where X is the proportion of the initial biomass remaining at time t and k is the decay constant (Wieder and Lang 1982) using nonlinear least-squares fitting. Stepwise multiple regression with backward elimination was used to identify how well initial root tissue chemical characteristics and root pigmentation class explained variation in k among root classes. Prior to regression, we tested for significant autocorrelation among initial root characteristics by pairwise correlation ($P < 0.05$ and $r > 0.80$). Significance of k and remaining root mass was tested among root classes and species (both categorical variables) with an analysis of covariance (ANCOVA) with time (continuous variable) as the covariate. All statistical analyses were conducted using JMP statistical software (version 7.0; SAS, Cary, North Carolina, USA).

Results

Root order

As expected, the initial chemical composition differed among roots of different order prior to their placement in litter bags (Table 1). Nutrient concentrations were generally higher in [1 + 2 B] roots than [3 + 4 B] roots. For example, N concentration in lower-order [1 + 2 B] roots was almost twice that of higher-order [3 + 4 B] roots for *A. pseudoplatanus*, *T. cordata*, and *L. decidua*,

TABLE 2.   Probability values from an analysis of variance (ANOVA) of root order on initial tissue chemistry at start of litterbag experiment in four temperate tree species of initial chemical concentrations or ratios.

Factor	C	N	Lignin	Al	Ca	Mg	P	C:N	Lignin:N	Lignin:P	Ca:Al
Species	0.0006	<0.0001	*0.05*	<0.0001	<0.0001	0.003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Root order	0.62	<0.0001	0.97	0.055	0.22	<0.0001	0.41	<0.0001	<0.0001	0.039	<0.0001
Species × root order	0.033	<0.0001	0.92	0.011	<0.0001	<0.0001	<0.0001	<0.0001	0.0009	0.041	<0.0001

Note: Roots were of two root order classes: pigmented, first- and second-order roots [1 + 2 B] and pigmented third- and fourth-order roots [3 + 4 B] of four temperate tree species (*Acer pseudoplatanus* L., *Tilia cordata* Mill., *Larix decidua* Mill., and *Pinus sylvestris* L.; $n = 3$ root litterbags per sampling time). Boldface indicates $P < 0.05$; italics indicate $P < 0.10$.

TABLE 1. Extended.

C:N	Lignin:N	Lignin:P	Ca:Al
31 (0.8)	14 (0.7)	127 (13)	5 (0.2)
50 (2.7)	23 (5.1)	138 (23)	16 (1.6)
30 (0.9)	11 (3.7)	93 (30)	9.9 (0.5)
56 (1.7)	28 (7.6)	213 (47)	14 (0.8)
23 (0.1)	9 (1.5)	33 (6)	0.82 (0.1)
41 (0.8)	16 (4.5)	78 (24)	0.90 (0.03)
24 (1.0)	6 (1.4)	18 (5)	0.74 (0.03)
25 (0.9)	7 (1.0)	24 (4)	1.23 (0.03)

but not for *P. sylvestris* (species × root-order interaction, $P < 0.0001$). Of the chemical characteristics measured, lignin was not affected by root order (Table 2; order effect, $P > 0.97$ or species × order effect, $P > 0.92$). In general, *P. sylvestris* showed the smallest differences in tissue chemistry among root orders. When comparing the initial chemistry of the roots placed in the litterbags that were derived from nursery trees (Table 1) with that of the trees in the common garden (Appendix B), concentrations of N, P, Ca, and Mg were generally higher and Al concentration lower in the roots of the nursery seedlings. Patterns of higher N and P concentrations in lower-order than higher-order roots observed in the nursery seedlings, however, were similar to those occurring in the roots of the common garden trees.

Initial tissue chemical parameters of the litter bag roots were more highly intercorrelated with decomposition for lower-order (first- and second-order) roots than for higher-order (third- and fourth-order) roots (Appendix C). In third- and fourth-order roots, many chemical parameters were significantly correlated. For example, initial Mg concentration was positively correlated to initial N ($r = 0.93$), P ($r = 0.94$), Al ($r = 0.80$), and Ca ($r = 0.84$) and negatively correlated with Ca:Al ratio ($r = -0.73$). However, in first- and second-order pigmented roots, Mg was negatively correlated with N ($r = -0.74$), P ($r = -0.84$), and Al ($r = -0.85$) but was positively correlated with Ca:Al ratio ($r = 0.58$).

Despite their higher C:N ratio and larger diameter (lower surface : mass ratio), higher-order [3 + 4 B] roots decomposed faster than lower-order [1 + 2 B] roots, as reflected by significant time, order, or order × time effects (Fig. 1). This was true under their own- and common-species canopies. Under their own-species canopy, *A. pseudoplatanus* and *T. cordata* showed the greatest differences in rates of decomposition between root-order classes. Higher-order [3 + 4 B] roots of both species lost ~2.5 times as much of their initial mass than lower-order [1 + 2 B] roots. In contrast, *L. decidua* mass loss of higher-order [3 + 4 B] roots was 1.75 times greater than in lower-order [1 + 2 B] roots, with a difference of only 15% between root orders for *P. sylvestris*. Under
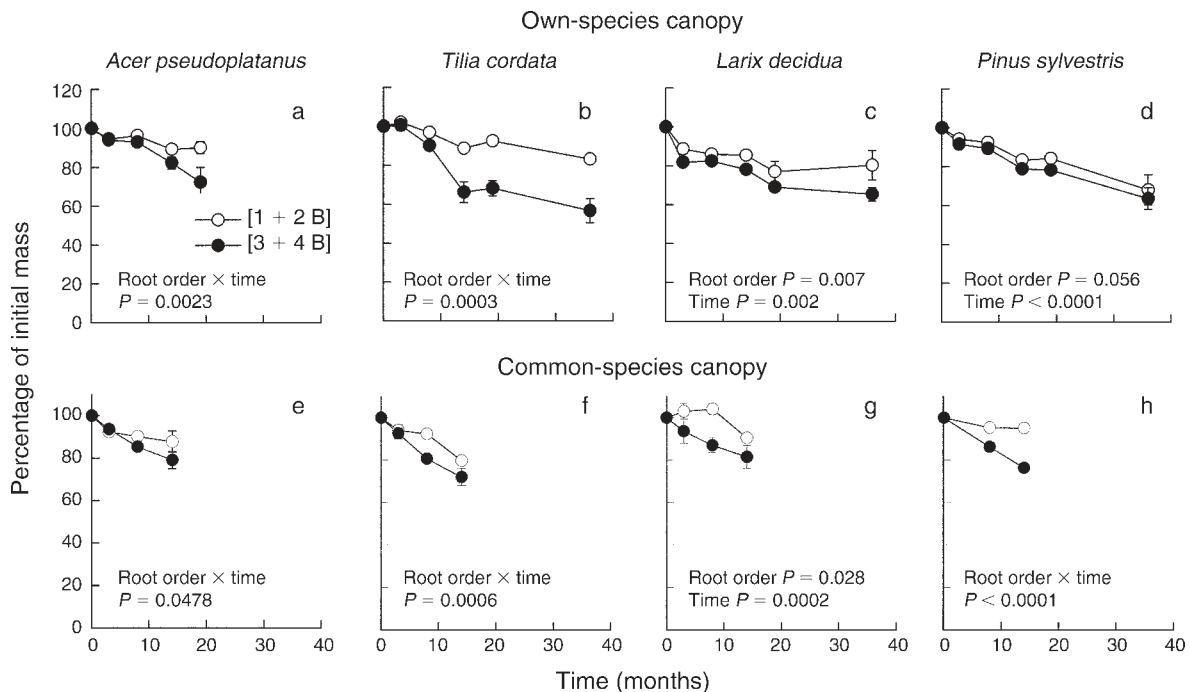

FIG. 1. Effects of root branching order on biomass during decomposition. Data are expressed as percentage of initial mass under the (a–d) own-species canopy and (e–h) a common-species canopy for four tree species. Pigmented first- and second-order roots [1 + 2 B] and pigmented third- and fourth-order roots [3 + 4 B] represent roots of different root order class, from root litterbags placed at 10-cm soil depth and harvested after 3, 8, 14, 19, and 36 months for the own-species canopy placement and after 3, 8, and 14 months under the common-species canopy placement. Error bars represent ±SE ($n = 3$ root litterbags per sampling time, including the different root classes). The experiment was established in the common garden "Morawina" of the Siemianice Experimental Forest near the village of Biadaszki in southwestern Poland.
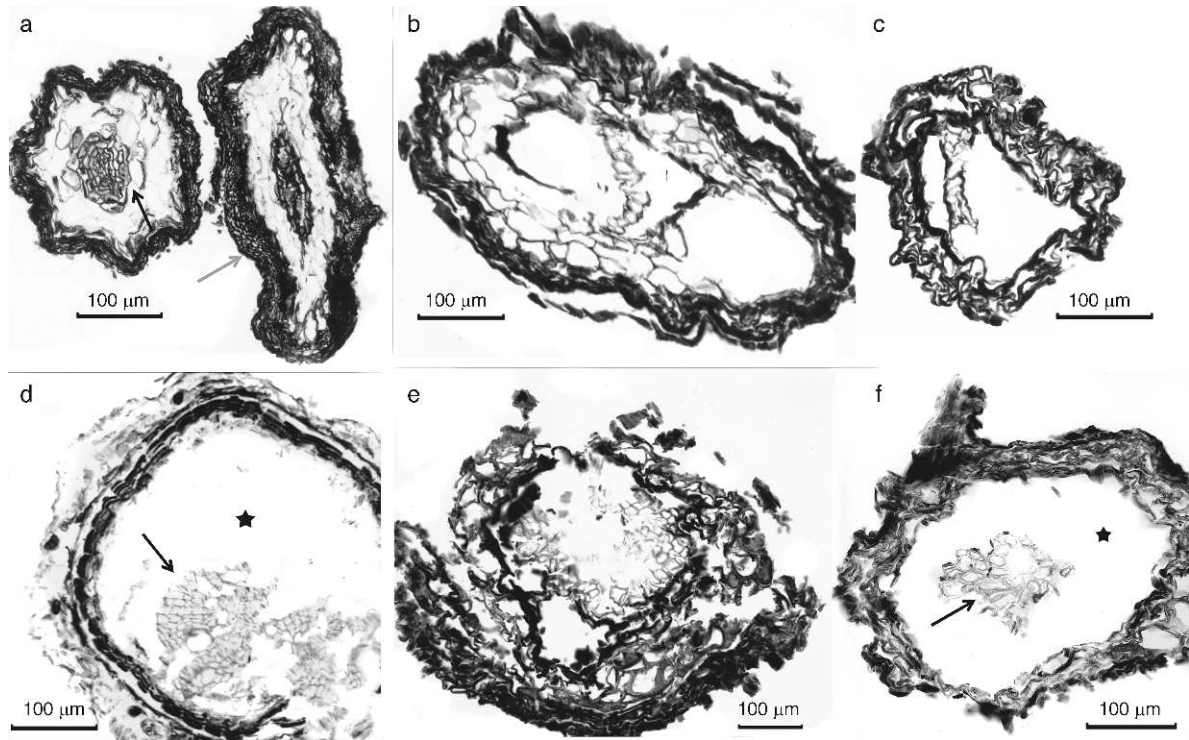
FIG. 2. Cross sections of pigmented (a–c) first- and second-order roots and (d–f) third- and fourth-order roots of *Tilia cordata*, *Larix decidua*, and *Pinus sylvestris* (left to right) after 36 months of decomposition. (a) *Tilia cordata* first- and second-order roots exhibited intact cell walls of endodermis (black arrow) and thick mycorrhizal fungal mantle (gray arrow). (d, f) *Tilia cordata* and *Pinus sylvestris* third- and fourth-order roots had only remnants of xylem elements (arrow) and no cell walls visible between the outer part of bark and xylem remnants (star). (b, e) *Larix decidua* shows an intermediate state of decay of both root orders.

the common-species canopy, a similar significant effect of root-order mass loss was observed for *A. pseudoplatanus*, *T. cordata*, and *L. decidua*, while *P. sylvestris* showed a more significant order × time effect for the initial 14 months, in contrast to its mass loss under its own-species canopy.

Anatomical transverse cross sections of root-order segments in the litterbags of all four species after 19 months of decomposition revealed relatively high structural integrity in both the lower and higher-order roots (Appendix D). In contrast, after 36 months of decay, many root structures had clearly decomposed (Fig. 2). In *Tilia*, structures in roots of the [1 + 2 B] root class were reasonably intact, while the [3 + 4 B] root class showed considerable degradation of the secondary xylem (Fig. 2a, d). In cross sections of *Larix* and *Pinus* roots, structural loss was apparent in both root order classes after 36 months of decomposition.

The single, negative exponential model described the actual rates of mass loss well for roots of the two root order classes using own-species plots. The estimated mass remaining fit the actual mass remaining for [1 + 2 B] roots with an $R^2 = 0.56$ ($n = 69$) and in [3 + 4 B] roots with an $R^2 = 0.75$ ($n = 69$). The intercepts (b_0) of the regressions between the estimated and the actual mass

remaining underestimated the actual rate of decay ($b_0 = 5.5$ for [1 + 2 B] roots, $b_0 = 29$ for [3 + 4 B] roots). Over the time period of 36 months, the decomposition constant (k) was 0.10 for the [1 + 2 B] roots and 0.19 for [3 + 4 B] roots (Table 3). The predicted rates of decay for both the individual root order classes and the combined root order classes ([1 + 2 B] and [3 + 4 B]) were negatively related to initial Mg concentrations ($P < 0.001$) and positively related to initial P concentrations ($P < 0.001$), with R^2 ranging from 0.21 to 0.28. This was also the case when combining the root litter quality parameters with the categorical parameter root order class, which did not lead to an increase in the coefficient of determination ($R^2 = 0.28$). Initial root Mg was negatively correlated with initial root Ca:Al and positively correlated to initial root Al (Appendix C). However, exchanging Mg with Al greatly weakened the description of predicted rates of decay ([1 + 2 B], $R^2 = 0.07$; [3 + 4 B], $R^2 = 0.04$). Additionally, P and Mg were significant covariates when run in the ANCOVA model based on percentage mass loss (analyses not shown).

In contrast to root mass, root N content declined more rapidly in the lower-order [1 + 2 B] roots than in the higher-order [3 + 4 B] roots (Fig. 3), especially in earlier stages of decomposition under their own-species

TABLE 3. Final models resulting from stepwise regressions of the root decomposition coefficient, k, against root chemical characteristics by root order class.

Fine-root order classes	k	df	R^2	Model equation
Chemical properties only				
[1 + 2 B]	0.10	69	0.23	$k = 0.11 - 0.07 \times (\text{Mg}) + 0.02 \times (\text{P})$
[3 + 4 B]	0.19	69	0.28	$k = 0.25 - 0.17 \times (\text{Mg}) + 0.05 \times (\text{P})$
Combined	0.15	138	0.21	$k = 0.19 - 0.11 \times (\text{Mg}) + 0.03 \times (\text{P})$
Root order class + chemical properties	0.15	138	0.28	$k = 0.13 - 0.10 \times (\text{Mg}) + 0.38 \times (\text{P}) + 0.08 \times (\text{root order class})$

Notes: Results are separated into fine-root orders: pigmented first- and second-order roots [1 + 2 B]; pigmented third- and fourth-order roots [3 + 4 B]; first- and second- and third- and fourth-order roots (combined); and root order classes + chemical properties. All terms included in the models were significant at $P < 0.001$.

canopy. Moreover, in the higher-order roots, N increased in the first 10 months from ~110% of initial content in *P. sylvestris* to nearly 150% of initial content in *T. cordata*. Even after 19 months for *A. pseudoplatanus* and 36 months for *T. cordata*, *L. decidua*, and *P. sylvestris*, N content was similar to initial levels and generally similar between root order classes. Under the common-species canopy, N content showed a similar initial N increase in higher-order [3 + 4 B] roots as under the own-species canopy.

The effects of root order on patterns of nutrient loss of P, Mg, and Ca were not consistent across species or between common and own plots (Appendix E). In *A. pseudoplatanus* and *P. sylvestris*, root order had essentially no effect on P loss, whereas in *L. decidua*, loss was

generally more rapid in the [1 + 2 B] root orders. In *T. cordata*, the effect of root order on P loss reversed from common to own plot. In general, the Mg content declined rapidly and similarly in both root order classes over time under the own- and common-species canopy, with losses of 30–80% of initial Mg pools during the first three months of decay, under the own and common canopy, respectively. Calcium content was only affected by root order in *T. cordata*, with higher-order roots exhibiting greater accumulation than the lower-order roots over time.

Root pigmentation

In contrast to our expectations, several key aspects of initial root tissue chemistry were similar among first- and
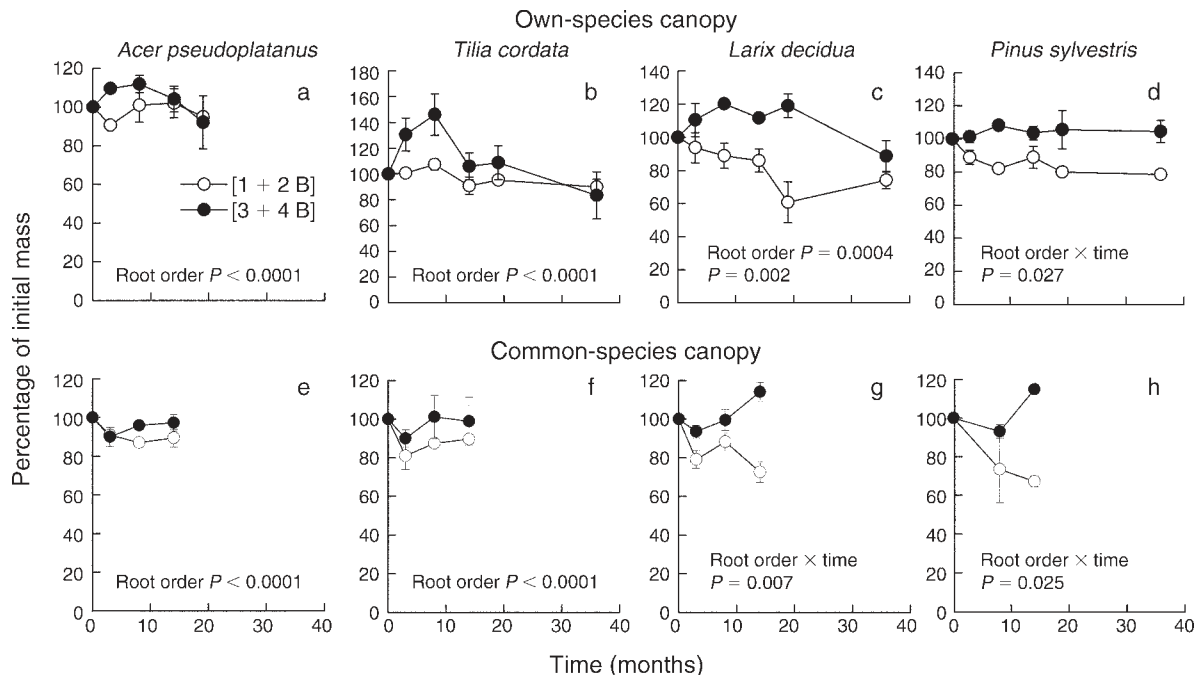

FIG. 3. Effects of root branching order on nitrogen content during decomposition. Data are expressed as percentage of initial nitrogen under (a–d) the own-species canopy and (e–h) a common-species canopy for four tree species. Pigmented first- and second-order roots [1 + 2 B] and third- and fourth-order roots [3 + 4 B] represent roots of different root order classes, from root litterbags placed at 10-cm soil depth and harvested after 3, 8, 14, 19, and 36 months for the own-species canopy placement and after 3, 8, and 14 months under the common-species canopy placement. Error bars represent ±SE ($n = 3$ root litterbags per sampling time, including the different root classes). Note different *y*-axis scales in panels (a)–(d).

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TABLE 4. The effects of root pigmentation on initial tissue chemistry at the start of the litter bag experiment in two temperate tree species.

Species	Root pigmentation	C	N	Lignin	Al	Ca	Mg
Acer pseudoplatanus	[1 + 2 W]	470 (3.8)	14.8 (0.9)	200 (17)	0.49 (0.0)	5.4 (0.4)	3.1 (0.3)
	[1 + 2 B]	488 (0.2)	15.8 (0.4)	227 (8.5)	1.15 (0.1)	6.2 (0.33)	3.1 (0.2)
Tilia cordata	[1 + 2 W]	479 (4.4)	15.5 (0.2)	289 (29)	0.61 (0.0)	4.1 (0.1)	2.7 (0.1)
	[1 + 2 B]	490 (2.0)	16.3 (0.5)	189 (54)	1.47 (0.1)	14.5 (0.7)	2.5 (0.1)

Notes: Data are expressed as milligrams per gram (with SE in parentheses; $n = 3$ root litterbags per sampling time) of root dry mass of two root pigmentation classes: white first- and second-order roots [1 + 2 W] and pigmented first- and second-order roots [1 + 2 B]; pigmented roots are older than white roots.

second-order roots of different pigmentation, [1 + 2 W] and [1 + 2 B] (holding order constant; Table 4). Several parameters linked to rates of tissue decomposition in other studies, including initial root N and lignin concentration as well as C:N and lignin:N ratios (Silver and Miya 2001), were not clearly affected by either root pigmentation across all species or even within single species (lack of interaction of species and pigmentation; Table 5). However, concentrations of some elements did differ between younger, white [1 + 2 W] and older, brown [1 + 2 B] roots. For example, initial Al concentration was much higher in pigmented roots. For other root tissue chemistry parameters, including initial concentrations of Ca and P and Ca:Al ratio, the relation to root pigmentation varied by tree species (Table 4). Pigmented roots of *T. cordata* had much higher Ca concentrations than white roots, whereas in *A. pseudoplatanus*, Ca concentration was similar in white and pigmented roots. Pigmented roots of *T. cordata* had lower P concentrations than white roots, but in *A. pseudoplatanus*, there was no change in P concentration with root pigmentation. In *T. cordata*, pigmented roots had higher Ca:Al ratios than white roots, whereas the pattern was reversed in *A. pseudoplatanus* (Table 4).

Despite few consistent differences in chemistry, white roots decomposed faster than pigmented roots of the same root order class as hypothesized. Over the 14-month observation period, white roots lost ~30% of their mass, while pigmented roots of the same root order lost only 10% of their mass (Fig. 4).

The single, negative exponential model described observed rates of decay well across root pigmentation classes, and there was no evidence of two distinct phases of decay during 14 months. The estimated mass remaining fit the actual mass remaining for [1 + 2 W]

roots with an $R^2 = 0.91$ ($n = 24$) and for [1 + 2 B] roots with an $R^2 = 0.64$ ($n = 24$). The rate of decay was underestimated for white roots, as indicated by the intercept of $b_0 = 16$, but estimated well for pigmented roots ($b_0 = 0.5$). Over the time period of 14 months, the average decomposition constant (k) was 0.28 for [1 + 2 W] roots ($R^2 = 0.72$) and was correlated positively with initial Ca ($P = 0.0002$) and lignin:P ratio ($P = 0.0011$) and negatively with initial Mg ($P = 0.0031$; Table 6). Despite the tight correlation between initial root Mg and N (Appendix F), replacement of Mg by N in the model did not improve prediction of decomposition rates ($R^2 = 0.61$). In contrast, the decay constant (k) was much lower in [1 + 2 B] roots ($k = 0.08$, $R^2 = 0.49$). Decomposition in the pigmented roots was positively related to initial root C:N ratio ($P = 0.001$) and negatively related to initial Mg concentration ($P = 0.0016$). When both root pigmentation classes were analyzed together ($k = 0.15$, $R^2 = 0.47$), decay rate was negatively related to initial Mg concentration ($P = 0.0022$) as well as to initial N ($P = 0.031$) and Al ($P = 0.028$) concentration (Table 6). Excluding or replacing initial root Mg with initial root N or Ca in the combined model did also not improve prediction of decomposition rates ($R^2 = 0.35$ in both cases). The combination of root tissue chemistry parameters (lignin:P, $P < 0.0009$; N, $P < 0.002$; Ca, $P < 0.034$) and the categorical parameter, root pigmentation class ($P < 0.0031$), predicted variation in the decomposition constant ($k = 0.15$, $R^2 = 0.52$) better than the chemical parameters alone, except for [1 + 2 W] roots, suggesting that the chemical properties measured could not fully account for variation in tissue quality associated with root pigmentation.

In contrast to the more rapid biomass loss in white than pigmented roots across the two species, N content

TABLE 5. Probability values from an ANOVA of root pigmentation on initial tissue chemistry at the start of the litterbag experiment in two temperate tree species of initial chemical concentrations or ratios.

Factor	C	N	Lignin	Al	Ca	Mg	P	C:N	Lignin:N	Lignin:P	Ca:Al
Species	*0.07*	0.36	*0.09*	0.33	*0.08*	0.12	**0.001**	0.59	0.18	0.99	0.72
Root pigmentation	**0.003**	0.26	0.57	**0.0005**	0.30	0.92	0.51	0.62	0.78	0.72	**<0.0001**
Species × root pigmentation	0.24	0.88	*0.08*	0.27	**<0.0001**	0.54	**0.047**	0.95	0.18	0.515	**<0.0001**

Notes: Roots were of two root pigmentation classes: white first- and second-order roots [1 + 2 W] and pigmented first- and second-order roots [1 + 2 B] of two temperate tree species (*Acer pseudoplatanus* L. and *Tilia cordata* Mill.; $n = 3$ root litterbags per sampling time). Boldface indicates $P < 0.05$; italics indicate $P < 0.10$.

TABLE 4. Extended.

P	C:N	Lignin:N	Lignin:P	Ca:Al
1.7 (0.1)	31 (1.8)	14 (2)	118 (3.8)	11 (0.7)
1.8 (0.1)	31 (0.8)	14 (0.7)	127 (13)	5.3 (0.2)
2.5 (0.1)	31 (0.6)	18 (1.8)	117 (17)	6.8 (0.5)
2.0 (0.1)	30 (0.9)	11 (3.7)	93 (30)	9.9 (0.5)

decreased more rapidly only in white roots of *T. cordata*, but not in those of *A. platanoides* (Fig. 4). In addition, no effect of pigmentation was observed on loss of Mg and P over time ($P > 0.77$; Appendix G). Only in *T. cordata* did pigmentation influence root Ca content over the 14 months, with white roots showing a rapid increase of 230% whereas pigmented roots remained essentially unchanged.

DISCUSSION

The roots with the greatest likelihood of dying (i.e., with the fastest turnover) are first- and second-order roots (Eissenstat et al. 2000, Wells and Eissenstat 2001, Guo et al. 2008*a*, *b*), yet decomposition of these particular root orders has rarely been studied. Some studies have examined decomposition (disappearance) using minirhizotrons, but the effect of different root types (white vs. pigmented, roots of different order) were not addressed (Comas et al. 2000, Ruess et al. 2003). Our analysis of root decomposition, based on differences in structure and function among finest root orders, revealed that among the species studied here, decay rates in these lowest orders were significantly slower than those of higher root orders. These results are in agreement with Fan and Guo (2010), who examined decomposition of different root orders in *Larix gmelinii* and *Fraxinus mandshurica*. After 17 months, they observed ~20–30% mass loss in first- and second-order

roots in the two species and ~20–40% greater mass loss in the third- and fourth-order roots. Other studies that used diameter classes have also indicated that the finest roots decomposed at a slower rate than somewhat coarser roots (McClaugherty et al. 1984, diameter 0–0.5 mm and 0.5–3 mm; Fahey et al. 1988, diameter 0.6–1 mm and 1–2.5 mm; Lõhmus and Ivask 1995, diameter <1 mm and <2 mm). Yet, in many species, even bulk fine-root samples of a root diameter <0.5 mm can contain at least three root orders, with mass biased toward the higher-order roots (Pregitzer et al. 2002). Careful selection of diameter classes to represent different groups of root orders is possible (e.g., Withington et al. 2006) and might be an alternative approach to distinguishing roots of different function; however, it is not clear that the early studies chose their diameter classes along these functional criteria. Where functional criteria are not used, our study suggests that bulk root decomposition studies may overestimate rates of decomposition of the roots that most frequently die. In addition, nutrient dynamics of first- and second-order roots may differ substantially from those of third- and fourth-order roots, especially in the early phases of decomposition: we found more rapid nitrogen loss (despite slower mass loss) in lower-order than in higher-order roots. A more mechanistic understanding of root decomposition and its contribution to ecosystem carbon and nutrient dynamics requires a fundamental shift in experimental methods toward stratifying root samples for decomposition studies along more functionally based criteria such as root order coupled with root-order-specific understanding of root mortality.

The patterns of decomposition in the different root order classes were opposite what might be expected from C:N ratio or root diameter. For most species in our study, initial litter chemistry differed among root orders in a manner similar to what has been previously reported (Pregitzer et al. 2002, Guo et al. 2004, Wang
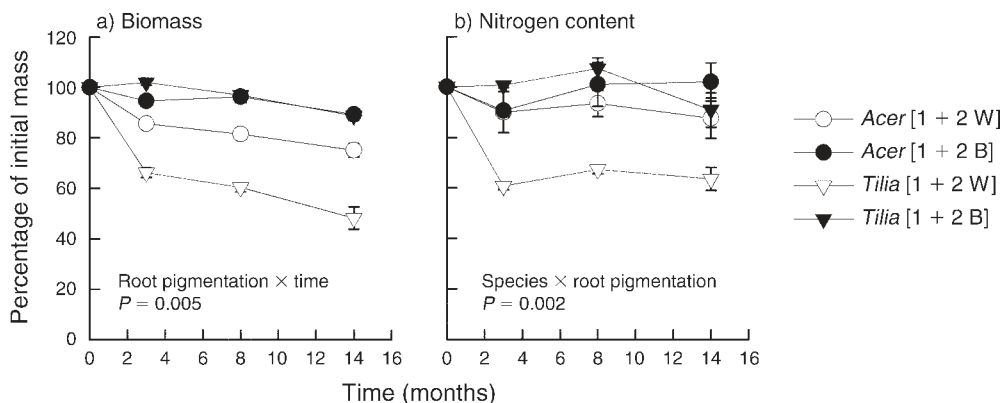

FIG. 4. Effects of root pigmentation on (a) biomass and (b) nitrogen content during decomposition. Data are expressed as the percentage of initial mass or nitrogen under the own-species canopy only, contrasting younger white first- and second-order roots [1 + 2 W] with older pigmented first- and second-order roots [1 + 2 B] in two temperate tree species (*Acer pseudoplatanus* and *Tilia cordata*) over a time period of 14 months. Error bars represent ±SE ($n = 3$ root litterbags per sampling time, including the different root classes).

TABLE 6. Final models resulting from stepwise regressions of the root decomposition coefficient, k, against root chemical characteristics by root pigmentation class for roots in "own" plots.

Fine-root pigmentation classes	k	df	R^2	Model equation
Chemical properties only				
[1 + 2 W]	0.28	23	0.72	$k = -0.32 + 0.06 \times (Ca) + 0.002 \times (lignin{:}P) - 0.08 \times (Mg)$†
[1 + 2 B]	0.08	23	0.49	$k = -0.43 + 0.01 \times (C{:}N) - 0.06 \times (Mg)$‡
Combined	0.15	47	0.47	$k = 0.73 - 0.1 \times (Mg) - 0.07 \times (Al) - 0.02 \times (N)$
Root pigmentation class + chemical properties	0.15	47	0.52	$k = 0.32 + 0.001 \times (lignin{:}P) - 0.03 \times (N)$ $- 0.18 \times (root\ age\ class) + 0.01 \times (Ca)$

Notes: Results are separated into fine-root pigmentation classes: white first- and second-order roots, [1 + 2 W], or pigmented first- and second-order roots, [1 + 2 B], or white and pigmented first- and second-order roots (combined) or root pigmentation classes + chemical properties. All terms included in the models were significant at $P < 0.001$. See *Methods: Plant material* for a description of "own" plots.

† The parameters in the equation could be replaced with (in this order) Ca, Mg, and P and result in an R^2 of 0.70.

‡ The parameter Mg in the [1 + 2 B] equation could be replaced with the parameter P remaining negatively related and result in an R^2 of 0.47.

et al. 2006, Fan and Guo 2010) with lower C:N ratio, lignin:N, and lignin:P in finer, lower-order [1 + 2 B] roots compared to coarser, higher-order [3 + 4 B] roots. However, [3 + 4 B] roots with higher C:N, lignin:N, and lignin:P ratios decomposed faster than [1 + 2 B] roots with lower values of these ratios in three of four species, which is in contrast to past syntheses of leaf litter decomposition (Cornwell et al. 2008).

Initial chemical parameters only partially explained the variation in decomposition among root orders. When Mg and P were used as covariates in an ANCOVA of mass loss, root order and the interaction of species and order were still highly significant. One possible explanation for the relatively greater recalcitrance of the first- and second-order, pigmented roots to microbial decomposition is that these roots are typically heavily colonized by mycorrhizal fungi, which may have important chemical consequences for decomposers that are not captured by typical measures (Langley and Hungate 2003, Guo et al. 2008a, Valenzuela-Estrada et al. 2008). Our study species included both arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) tree species (Withington et al. 2006). Both types of mycorrhizal fungi produce the compound chitin, which may slow rates of decomposition, as found in bulk fine roots (diameter <1 mm) (Langley et al. 2006), despite being rich in N. Whether chitin or some other chemical aspect of mycorrhizal fungi that transcends mycorrhizal type (AM vs. ECM) is the cause for the slower rates of decomposition in pigmented first- and second-order roots than white roots or higher-order roots needs further study, but chitin could be responsible for the relationship between decomposition rate and root C:N ratio being opposite of what has been seen previously for leaf litter. Besides chitin, mycorrhizal colonization can also increase defense-related, secondary antifungal metabolite concentrations (Gadkar et al. 2001, De Deyn et al. 2009). The increase in defense-related compounds could explain the intact root structures in cross sections of pigmented first- and second-order root of *T. cordata*, colonized by ectomycorrhizal fungi, in contrast to the more degraded structures in third- and fourth-order roots after 36 months of decay (Fig. 2).

Another factor that may contribute to the faster decomposition in the higher-order roots is their typically greater role in storage of carbohydrates. Fan and Guo (2010) observed an approximately 10–30% higher relative concentration of total nonstructural carbohydrates (TNC) in the third- and fourth-order roots than in the first- and second-order roots, depending on species. Within approximately two months of decomposition, TNC concentrations declined by >50–80% and no longer differed among orders. These easily degradable sources of carbon may have primed the decomposer community, causing continued faster decomposition in the higher-order roots.

The heterogeneous pattern of N mineralization and immobilization from fine roots of both groups of root classes contrasted with reported findings that root N is released linearly during the initial phases of decomposition in root tissues that have C:N ratios as high as 50 (Parton et al. 2007). In contrast, we found in our study that pigmented higher-order [3 + 4 B] roots with an initial C:N ratio ranging from 25 to 50 exhibited N immobilization. The root classes of lower-order [1 + 2 W] and [1 + 2 B] roots (C:N ranging from 24 to 30) exhibited either very little change in N content or N mineralization. In contrast, Fan and Guo (2010) observed in a relatively nutrient-rich soil in northeastern China that all root orders initially lost N in the first two months, but the highest-order roots immobilized N to varying extent in the following months of decomposition. Temporary immobilization of root N was also reported in decaying roots with diameters less than 1 mm (Lõhmus and Ivask 1995) and less than 2 mm (Ostertag and Hobbie 1999, Guo et al. 2006, Langley et al. 2006), indicating that bulk fine-root samples may exhibit net release of N at lower C:N ratios than roots separated by order, at least at sites of low soil fertility. One possible explanation for N immobilization in the early phase of decomposition of higher-order [3 + 4 B] roots in this study is that these roots may have a large

fraction of their C compounds in the form of readily degradable starch (Fan and Guo 2010), leading to appreciable mass and C loss and the immobilization of some microbial N, especially of chitin-rich decomposer fungi. In lower-order roots, there is much less starch and more protein N for nutrient assimilation. These differences in chemistry of different root orders underscore the importance of branching architecture in studies of root decomposition.

The influence of soil environment, as reflected by comparisons of common- vs. own-species plots, had only modest effects on the patterns of mass and N in relation to root order among species. Soils of *Pinus* and *Larix* plots differed appreciably from those of the common *Acer* plot. In *P. sylvestris*, there was evidence of faster decomposition and greater N immobilization in the higher root orders in the less acidic common plot than in the *Pinus* plot, possibly a result of higher microbial activity in the common plot.

Among the chemical parameters influencing mass loss of the different root orders, initial Mg concentration was often correlated with the decay constant. Initial root Mg concentration probably did not directly influence decomposition but was correlated with a chemical parameter that did, such as its general negative correlation with Ca:Al ratio (Appendix C). Thus, low Ca:Al ratio might more likely be the reason for inhibited microbial activity and slower rates of decomposition in some species, as found by Vogt et al. (1987). Initial P concentration and its influence on decomposition rates were expected, since many studies had already shown the importance of this parameter on the decay of leaf litter (Berg et al. 1993). Because root Mg and P concentrations as well as root order were significant ($P < 0.001$ for all three effects, Table 4), we conclude that these nutrients were contributing to variation in percentage mass loss largely independent of some other factor that was associated with root order that also influenced decomposition.

Even within root orders, additional factors besides root chemistry may contribute significant variation to root decomposition. Root pigmentation did not influence nutrient or lignin content among white and pigmented first- and second-order roots but did result in different decomposition rates. Young, white roots consistently had faster rates of decomposition than older, pigmented roots of the same order. Since pigmentation is associated with accumulation of condensed tannins and other accumulated defenses (Wells and Eissenstat 2003), white roots may be readily consumed by microbial decomposers. For instance, a recent study showed that herbivores such as Collembola predominately feed on white fine roots and root hairs, rather than soil microbes (Endlweber et al. 2009). Furthermore, when estimates of root mortality, such as those derived using minirhizotrons, are combined with estimates of decomposition, it may be important to distinguish between the populations of roots that are young and still white at the time of death from the remainder of the root population that is pigmented at death. Where white roots die (Wells and Eissenstat 2001, Anderson et al. 2003), the cycling of C and nutrients is likely to be rapid. In general, it is not clear whether the delay in pigmentation of the youngest and most vulnerable roots is an unavoidable consequence of development, or whether, as hypothesized for some tropical forest leaves that also delay pigmentation, expensive chemical investment is delayed until roots become slightly more physically recalcitrant and thus less physically vulnerable (Kursar and Coley 1992).

Root pigmentation or root order always described a substantial portion of the variance in the rate of percentage mass loss when the other chemical traits were used as covariates, indicating key factors associated with the influence of pigmentation and order on decomposition were not measured. Other chemical and structural properties such as concentrations of non-structural carbohydrates, soluble phenolics, and chitin need to be explored to better understand more mechanistically how root pigmentation and order affect decomposition. Still, some of our findings are consistent with results of a study at the same site that compared decomposition of leaf litter with that of bulk fine roots (diameter <2 mm) across 11 species (Hobbie et al. 2010). They found that species with higher specific root length (SRL; root length per unit dry mass) (thinner) roots decomposed more slowly than species with lower SRL (thicker) roots, similar to our findings for roots of different order within the same species.

While the present study clearly shows the relative importance of root pigmentation and root order on decomposition, absolute decomposition rates in the field may differ from what are reported here. The use of dried fine roots can lead to overestimates of rates of decomposition and nutrient cycling (Ludovici and Kress 2006). In addition, the use of mesh litter bags allowed only microbial decay and resulted in disturbed rhizospheres, likely leading to underestimates of rates of root decomposition (Dornbush et al. 2002), especially for the vulnerable white first- and second-order roots (Pregitzer et al. 1997, Stevens et al. 2002, Withington et al. 2006). The loss of only 20% of first- and second-order root mass after 36 months in several of the species in this study, for example, may underestimate root decomposition in situ where soil fauna and microbial-rich rhizospheres contribute to decomposition. Further study is necessary to determine to what extent this might bias our observations that the lower root orders decomposed slower than the higher root orders or that white roots decompose faster than pigmented roots. Lastly, the use of nursery-grown seedlings also might affect absolute patterns but not likely relative patterns in at least nutrient concentrations between root order classes.

In summary, root decomposition can be influenced by root pigmentation and root order, but previous root decomposition studies have rarely considered this fine-

scale heterogeneity in fine roots. Pigmented first- and second-order roots that can account for ~75% of the total length of fine roots (Pregitzer et al. 2002) may decompose more slowly but lose elements such as N more readily compared to the higher-order third- and fourth-order roots. These differences were generally consistent across diverse species. While it is logistically much easier to include all roots below a certain diameter (e.g., <2 mm) as a single class, this approach masks important differences in tissue chemistry and known differences in the relative contribution of different root orders to the detrital pool (Pregitzer et al. 2002, Guo et al. 2008a, b, Valenzuela-Estrada et al. 2008). Moreover, classical hypotheses about the chemical controls of decomposition dynamics were in some cases opposite to observed responses. Thus, there is always a trade-off between gaining functional information that comes from analyzing roots of different orders and the enormous labor required to separate roots at this level. In order to understand the contributions of root turnover to C and nutrient cycling, we need to improve our understanding of not only the rates of root turnover of different root orders, but also how the different root classes decompose.

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APPENDIX A

Percentage of ectomycorrhizal morphotype colonization present on root systems of three temperate tree species (*Tilia cordata* Mill., *Larix decidua* Mill., and *Pinus sylvestris* L.) collected from local nurseries on the root order class pigmented, lower first- and second-order fine roots, by the Rudawska Laboratory, Institute of Dendrology, Kornik, Poland (*Ecological Archives* M081-004-A1).

APPENDIX B

Initial tissue chemistry of pigmented first- and second-order fine roots [1 + 2 B] and third- and fourth-order fine roots [3 + 4 B] from 1-yr-old ingrowth cores (August 2007) from three plots of each of four temperate tree species (*Acer pseudoplatanus* L., *Tilia cordata* Mill., *Larix decidua* Mill., and *Pinus sylvestris* L.) at the common garden "Morawina" in the Siemianice Experimental Forest, Poland (*Ecological Archives* M081-004-A2).

APPENDIX C

Correlation matrices of initial root tissue chemistry for pigmented first- and second-order roots, pigmented third- and fourth-order roots, and combined pigmented first-, second-, third-, and fourth-order roots of the root-order experiment (*Ecological Archives* M081-004-A3).

APPENDIX D

Cross sections of pigmented first- and second-order roots and third- and fourth-order roots of *Acer pseudoplatanus*, *Tilia cordara*, *Larix decidua*, and *Pinus sylvestris* after 19 months of decomposition (*Ecological Archives* M081-004-A4).

APPENDIX E

Effect of root branching order on P, Mg, and Ca content during decomposition under "own" and common species canopies (*Ecological Archives* M081-004-A5).

APPENDIX F

Correlation matrices of initial root tissue chemistry for white first- and second-order roots and pigmented first- and second-order roots of the root pigmentation experiment (*Ecological Archives* M081-004-A6).

APPENDIX G

Effects of root pigmentation of first- and second-order roots on P, Mg, and Ca content during decomposition under the "own" canopy species (*Ecological Archives* M081-004-A7).