Short report

Response of microbial respiration from fine root litter decomposition to root water content in a temperate broad-leaved forest

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Received on March 11, 2013; Accepted on May 29, 2013

Abstract: Microbial respiration from plant litter decomposition is sensitive to soil water status; however, its response to water status remains ambiguous, particularly in the litter of fine roots. We investigated the effect of fine-root water content on microbial respiration after 468 days of decomposition in forest soil for two diameter classes (0–0.5 and 0.5–2 mm) of *Quercus serrata* and *Ilex pedunculosa* in central Japan. Direct measurement of microbial respiration from root litter resulted in a range of 0.015–3.52 nmol CO_2 g^{-1} s^{-1}. Microbial respiration in both diameter classes and species decreased linearly with decreasing root water content. These changing patterns of microbial respiration did not differ significantly between the diameter classes of either species, indicating that microbial respiration was regulated by the moisture of root litter, and not by characteristics associated with diameter class or species. In contrast, the carbon to nitrogen ratio and mass loss of the root litter differed significantly between diameter classes in both species. These findings suggest that along with chemical and morphological properties of fine root litter, the changes in root water content should also be considered as a viable factor in microbial activity variations. Drying–wetting cycles of fine roots could lead to sensitive responses of microorganisms during the short term, leading to variation in the decomposition rate of fine root litter over the long term. This study provided insight into the potential impact of microbial physiological performance on heterotrophic respiration and fine root decomposition under the varying root water content.

Keywords: CO_2 gas-exchange measurement, fine root decomposition, heterotrophic respiration, microbial activity, root diameter, root moisture

Introduction

Microbial respiration (R_m) is a crucial component of soil CO_2 efflux associated with mineralization of dead organic matter via metabolism by fungi and bacteria (Raich and Schlesinger 1992, Couteaux et al. 1995). R_m is sensitive to variation in soil water status as water content strongly influences microbial physiological processes (Liu et al. 2002, Xu et al. 2004). When soil water content decreases, the metabolic activity of most microbial species also decreases, resulting in decreased R_m and nutrient mineralization (Griffin 1981, Schimel et al. 2007). Reportedly, the effect of water content on R_m differed among litter types both in laboratory experiments (Manzoni et al. 2012) and in the field (Jomura et al. 2012). Jomura et al. (2012) measured R_m of coarse woody debris and leaf litter in a broad-leaved forest. They suggested that sensitivity of microorganisms to changes in water status differs according to the type of dead organic matter. For accurately assessing R_m response to water content, we need to understand the difference in R_m values due to litter of leaf, branch, and root, and soil organic matter with water content under field conditions. One of the R_m sources from soil is fine root litter. Fine roots (<2 mm) are dynamic and short-lived, supplying much belowground litter input and accounting for up to 75% of net primary production of an ecosystem (Finér et al. 2011, Brunner et al. 2012). Thus, fine roots play an important role in carbon and nutrient accumulation in soil. However, despite the potential importance of R_m in fine root litter, there is still limited information on

Kawamura A, Makita N, Osawa A 2013 Response of microbial respiration from fine root litter decomposition to root water content in a temperate broad-leaved forest. Plant Root 7:77-82. doi:10.3117/plantroot.7.77

rapid response of CO_2 gas-exchange from root litter. Furthermore, the R_m response of decomposing fine root litter to the water content remains ambiguous.

Chen et al. (2000) reported that R_m from dead coarse roots (10–30 mm diameter) was significantly influenced by root water content and that the R_m asymptotically increased with increasing root water content. They also suggested that tree species and decomposition stage influenced R_m response to root water content. Recent studies reported that root systems are composed of individual roots with heterogeneous physiological and chemical properties (Hishi 2007, Makita et al. 2011). In fine roots, decomposition rate and limiting factors were different even among <2 mm diameter classes owing to different chemical and morphological properties (Fan and Guo 2010, Goebel et al. 2011). Root decomposition is a process in which micro-organisms mineralize root C and N to meet the balance of C and N in their biomass for optimizing their growth. Therefore the root C/N gives a good relationship to rate of root decomposition (Silver and Miya 2001, Berg and Meentemeyer 2002). In addition, several studies reported that finer roots decomposed more slowly than larger roots within 2 mm, because the finer diameter roots typically have higher N and P concentrations and acid insoluble fraction (Fan and Guo 2010, Goebel et al. 2011). Here we hypothesize that R_m response from fine root litter to root water content differs between roots of different diameter classes within the range of <2mm (Hypothesis 1). We also hypothesize that difference in C/N ratio of roots of different diameter classes leads to different R_m responses in relation to root water content (Hypothesis 2).

Our overall aim of the present study was to elucidate the microbial respiration of fine root litter decomposition and the pattern in microbial response to water status of fine roots. We investigated R_m in root litter in response to root water content in a temperate broad-leaved forest of central Japan, focusing on decomposition with respect to two diameter classes and C/N ratio of fine roots. First objective was to examine R_m response to temporary changes in root water content using fine root litter of *Quercus serrata* and *Ilex pedunculosa* after 468 days of decomposition, and the second was to determine whether R_m response to root water content differs between two root diameter classes (0-0.5 mm, 0.5-2 mm) and the litter C/N ratio.

Materials and Methods

Study site

This study was conducted at Ryukoku Forest, Otsu, Shiga, Japan (34°58′ N, 135°56′ E, 130 m a.s.l). Ryukoku Forest is a secondary broadleaved forest mainly of the canopy trees of *Quercus serrata* Thunb. and *Ilex pedunculosa* Miq. The soil of the study area is characterized by sand and small round gravels, classified as yellowish brown forest soil of the Kobiwako group derived from lacustrine sediments of Cenozoic origin (Ministry of Land Infrastructure Transportation and Tourism 1982). The mean annual precipitation is 1530 mm and temperature is 14.9 °C (1976–2011). The meteorological data for the site in Otsu-city were obtained from Japan Meteorological Agency.

Experimental setup for fine root decomposition

Fine roots of *Q. serrata* and *I. pedunculosa* were collected from upper 5 cm of mineral soil (A horizon) in May 2010. Living fine roots of both species were separated from soil particles, washed gently in deionized water, sorted into 0–0.5 and 0.5–2 mm diameter classes, and dried at 50 °C for 48 h. Approximately 0.4 g of dried root of each class was placed in a litterbag (10 × 5 cm) made of 0.85 mm nylon mesh.

We established one 10 × 10 m quadrat on a generally east-facing slope just below a ridge where *Q. serrata* and *I. pedunculosa* dominate. On 29 June 2010, 20 litterbags (2 species × 2 diameter classes × 5 replicates) were buried vertically in 0–5 cm depth in the mineral soil horizon. An additional set of six litterbags for each diameter class of each species was used to determine initial root chemistry and ash content. The root samples for measurements of these initial conditions are termed ‘initial roots’ in this study.

Measurement of microbial respiration and root water content

All litterbags were retrieved on 10 October 2011 (after 468 days of decomposition) for evaluating the response of sample roots which were being decomposed sufficiently by micro-organisms. The litterbags were transported to the laboratory within 2-hours of collection for respiration measurements. Remaining root litter was removed from the bags and gently hand-cleaned to remove soil particles. These roots are termed ‘decomposing roots’ thereafter. Rates of respiratory gas exchange as a specific R_m from the root litter (n = 5) were measured in a temperature-controlled room using a closed dynamic chamber system equipped with an infrared gas analyzer (LI-840, LI-COR, Lincoln, NE, USA), allowing respiration measurements to be performed within 5-hour of sample collection. Mean tempera-

ture of the sample roots was 24.0 ± 0.06 °C. We enclosed a root litter sample in the respiration chamber and monitored the CO_2 concentration in the chamber for 180 s. R_m was calculated from the slope of the linear increase of CO_2 concentration within the chamber. Further details of the measurement of root respiration are given in Makita et al. (2012).

To evaluate the respiration rate in response to root water content, the mass of the root sample under natural drying (W_{nd}, g) was measured in the respiration chamber. The set of procedures was repeated 13 times for each root sample at an interval of 30–120 min over 14 h. All samples were analyzed within a sampled date. Between each procedure, the moisture of root litter sample decreased spontaneously or gradually due to the natural drying. Thus R_m was measured repeatedly while the root water content was decreasing gradually. After respiration measurements, root samples were dried to constant mass at 50 ^{o}C and weighed (the root dry mass designated as W_d, g) Then the root water content at a given time during the experiment was calculated as $(W_{nd} - W_d)/W_d \times 100$ (%), where W_{nd} (g) is the fresh mass of the root sample that was measured at the beginning of the experiment. This respiration measurement and that of water content were similar to those of Chen et al. (2000).

Chemical analysis

The root samples used for the microbial respiration experiment above were thereafter milled for C and N analyses. Total C and N concentrations were measured using an NC analyzer (Sumigraph NC-900, Shimadzu, Japan). Ash content of the root samples for each diameter class of each species was determined by burning them at 550 °C for 4 h. All mass and C and N data for roots are expressed on an ash-free dry-mass basis except for the root water content.

Data analysis

All the carbon to nitrogen (C/N) ratios for initial and sampled root litter and mass remaining of sampled roots were arcsin-transformed for subsequent statistical analysis. Two-way ANOVA was used to compare differences in C/N for initial roots (before decomposition experiment) between species or root diameter classes as the main effect. Equality of the mean values of C/N and mass remaining of sampled root litter after 468 days of decomposition was also tested by two-way (species × diameter) ANOVA. Independent-sample t-tests were used to compare C/N differences for initial roots and sampled decomposing roots and differences in mass remaining for the decomposing roots between diameter classes for each species.

The effects of diameter classes on the relationship between R_m and root water content were tested with linear regression and analysis of covariance, using water content as the covariate. First, we tested for homogeneity of slopes between diameter classes within each species. Next, we used an analysis of covariance to test for equality of intercepts between the regression lines for two diameter classes. Multiple regression analysis was used to examine which variable (water content, diameter, and species) influenced the R_m. To determine which of the variables best explained the microbial respiration, the standard partial regression coefficient between the variables and R_m was calculated. Statistical analyses were performed with statistical software R 2.14.1.

Results

Characteristics of initial roots and decomposing roots

In the initial roots before decomposition, C/N ratio differed between diameter classes in both species (Table 1; $P < 0.001$). The C/N values of 0–0.5 mm roots of both species were approximately 60 % of those of 0.5–2 mm roots ($P < 0.001$).

In the decomposing roots (468 days of decomposition), the C/N also differed depending on diameter class and species (Table 1; $P < 0.001$). The C/N of 0–0.5 mm roots of both species was lower than those of 0.5–2 mm roots ($P < 0.05$). After 468 days of decomposition, the C/N of 0–0.5 mm roots decreased in *I. pedunculosa* ($P < 0.001$) but not in *Q. serrata* ($P = 0.14$). However, the C/N of 0.5–2 mm roots decreased in both species ($P < 0.001$). Mass remaining of decomposing root litter differed between the two diameter classes (Table 1; $P < 0.01$), but not between species (Table 1; Two-way ANOVA $P > 0.05$). Within species, mass remaining differed between diameter classes for *Q. serrata* ($P < 0.01$) but not for *I. pedunculosa* ($P = 0.62$). *Q. serrata* exhibited that percentage mass loss of 0–0.5 mm roots after 468 days of decomposition was lower than that of 0.5–2 mm roots.

Microbial respiration and root water content

Microbial respiration rates from root litter ranged from 0.015 to 3.52 nmol CO_2 g^{-1} s^{-1} (Fig. 1). The ranges of root water content for 0–0.5 mm and 0.5–2 mm roots were 24.4－305.7% and 19.3－288.0% for *Q. serrata*; and 13.1－267.4 % and 19.1－265.5% for *I. pedunculosa*.

Table 1. Carbon to nitrogen ratio (C/N) of initial root (n = 6) and C/N and mass remaining of decomposing root (n = 5) after 468 days of decomposition in two diameter classes (0–0.5 mm, 0.5–2 mm) of *Quercus serrata* and *Ilex pedunculosa* at Ryukoku Forest (mean with SE in parentheses). Same letters in the same column indicate that the means are not significantly different between two diameter classes in each species at the 5% level by the independent-sample t-test.

Diameter class	Initial root	Decomposing root	
	C/N	C/N	Mass remaining (%)
Quercus serrata			
0–0.5 mm	34.8 (1.2) a	31.9 (1.6) a	68.4 (3.2) a
0.5–2 mm	61.0 (2.5) b	37.8 (1.1) b	45.9 (2.8) b
Ilex pedunculosa			
0–0.5 mm	32.2 (0.8) a	24.2 (0.9) a	56.7 (3.8) a
0.5–2 mm	51.5 (2.3) b	38.8 (2.0) b	53.1 (2.0) a
P values			
Species	ns	**	ns
Diameter	***	***	**
Species × Diameter	ns	**	*

Significance in two-way ANOVA: ***P < 0.001; **P < 0.01; *P < 0.05; ns Not significant

There were significant correlations between R_m and root water content in each diameter class of both species (Fig. 1; P < 0.01), suggesting that R_m decreased gradually and possibly linearly with decreasing water content in both diameter classes for both species. Between the diameter classes, linear regression slopes did not differ significantly for either species (P > 0.05). Intercepts differed between the diameter classes for *I. pedunculosa* (P < 0.01), but not for *Q. serrata* (P > 0.05). Individual linear regression R^2 values of 0–0.5 mm and 0.5–2 mm roots were 0.80 and 0.77 for *Q. serrata* and 0.63 and 0.79 for *I. pedunculosa* (all P < 0.001). A multiple regression equation with standardized partial regression coefficient between R_m and three variables was as follows: $R_m = 0.185 + 0.790 \times$ water content $+ 0.232 \times$ species $- 0.088 \times$ diameter (R^2 = 0.73; P < 0:001). The standardized partial regression coefficient of water content, species, and diameter was 0.86, 0.17, and -0.065, respectively.

Discussion

CO_2 gas-exchange measurement is potentially of great value for evaluating immediate response of microbial activity on fine root litter. Microbial respiration from the root litter decomposition in two diameter classes of both *Q. serrata* and *I. pedunculosa* decreased linearly with root water content (Fig. 1). This result demonstrated that low moisture could reduce R_m rate from root litter by limiting the activity of decomposer micro-organisms. Chen et al. (2000) reported that decreasing root water content exerted a low-moisture limitation effect on R_m in coarse root litter of five conifer trees. We suggest that microorganisms sensitively respond to changing moisture content of substrates by adjusting their physiological activity and microbial biomass, thereby changing their metabolic activity and respiration rates.

Composition of the microbial community could also be a factor in the response of R_m to changes in water content (Fierer et al. 2009, Manzoni et al 2012). This compositional response is due to different sensitivities of fungi and bacteria to water stress. It is also due to a large variation in response to water stress even among species within the same fungal and bacterial groups (Brown 1990, Schimel et al. 2007). Our results suggest that the microbial communities of the root litter may be adjusted to produce similar R_m levels between diameter classes, despite the differences in chemical and morphological properties of the fine roots. Thus, the moisture content of root litter can be an important factor regulating R_m.

Our hypothesis 1 that R_m response to water content of fine root litter differs between roots of different diameter classes proved invalid for the classes of root diameters studied. In our study, litter properties of fine roots significantly varied between diameter classes (Table 1). The decomposing fine roots of two diameter classes had different characteristics in both species: 0–0.5 mm roots had lower C/N than 0.5–2 mm roots. Therefore, our Hypothesis 2 was also rejected. The difference in C/N ratio of fine roots of different diameter classes are similar to those found by other studies on fine root decomposition in woody species (Fan and Guo 2010, Goebel et al. 2011). Microorganisms mineralize C and N compounds with adjusting the balance of C and N in their biomass,

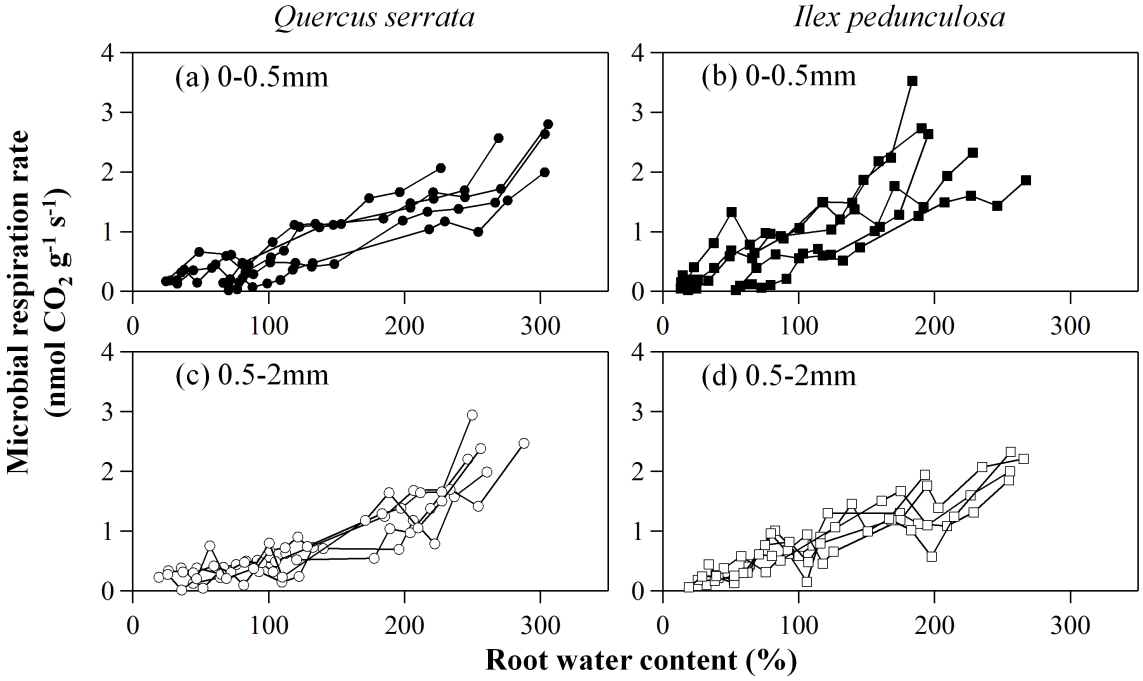

Fig. 1. Relationships between microbial respiration rate at 24.0 °C and root water content in fine root litter samples after 468 days of decomposition; (a: ●) 0–0.5 mm and (c: ○) 0.5–2 mm for *Quercus serrata* and (b: ■) 0–0.5 mm and (d: □) 0.5–2 mm for *Ilex pedunculosa*. The root water content during the experiment was calculated as $(W_{nd} - W_d)/W_d \times 100$ (%), where W_{nd} is root mass under natural drying, and W_d is oven-dry root mass at the end of experiment.

leading to mass loss and N dynamics of root litter through microbial decomposition. When microorganisms decompose higher C/N compounds, microorganisms re-immobilize mineralized N or immobilize N from exogenous environments (Parton et al. 2007, Manzoni et al. 2008). When microorganisms decompose lower C/N compounds below critical ratio which is determined by microbial C/N and C use efficiency, net N mineralization would occur (Manzoni et al. 2008). Thus decreasing C/N ratio in 0-0.5 mm root litters indicates that the decomposing substrate by microorganisms would affect microbial activity. On the other hand, the pattern of R_m change did not differ significantly between the diameter classes in either species, as R_m in both diameter classes responded similarly to root water content in both species. These results suggest that root water content is the most important factor determining microbial activity variations. In addition, on a basis of multiple regression analysis of R_m in relation to three explanatory variables (root water content, diameter, and species), root water content best explained the observed variations in R_m (72.2 %). These results suggest that along with chemical and morphological properties (C/N and diameter class) of fine root litter, root water content should also be considered as a viable factor in microbial activity variations.

This study provided the first attempt to quantify the rate of R_m in fine root litter by its immediate measurement with an experimental approach. The respiration measurement yielded quantitative data on the potential impact of microbial physiological performance on fine root decomposition under varying water contents of fine roots. Under field conditions, stochastic rainfall events may cause the soil water content to fluctuate widely over time, resulting in large variations in root litter water content. The net effect of drying–wetting cycles in substrate could lead to sensitive responses of microorganisms during short term and to variation in decomposition rate and physicochemical qualities of the root litter over long term. The sensitive response of R_m enables us to better understand heterotrophic respiration from litter of tree fine roots in forest ecosystems. However, the present data are limited to samples in a specific period (after 468 days of decomposition). There is evidence that early stage of decomposition is controlled primarily by concentrations of limiting nutrients, especially N, whereas lignin decomposition exerts dominant control in the later stages (Couteaux et al. 1998, Berg and McClaugherty 2007). It might be possible that response of R_m to material water content be altered during decay. Therefore, more work is required under natural forest conditions to determine linkages between the R_m sensitivity and water content of fine roots through drying–wetting cycles in different decomposition stages.

Acknowledgments

The authors acknowledge Y. Kosugi and M. Dannoura of Kyoto University for constructive suggestions on this study and T. Miyaura of Ryukoku University for allowing work at Ryukoku Forest. We also thank members of Forest Utilization laboratory, Kyoto University for assistance. This work was supported in part by the Sasakawa Scientific Research Grant from the Japan Science Society to A.K.

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