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An improved method for measuring the production, mortality and decomposition of extramatrical mycelia of ectomycorrhizal fungi in forests

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ABSTRACT

The production and mortality of extramatrical mycelia (EMM) of ectomycorrhizal fungi are poorly quantified despite their importance in soil carbon cycling in forests. Ingrowth bag/core methods are the most widely used but can not accurately assess temporal changes in EMM production and mortality, resulting in great uncertainty in annual estimates. A modified method using two mathematical models (Biomarker and Algebraic models) is proposed to quantify EMM production, mortality and decomposition over differing time periods by integrating EMM decomposition dynamics with ingrowth core/bag data. In the Biomarker model, EMM biomass and EMM total mass (sum of necromass and biomass) are assumed to be known by using chemical biomarkers as proxies. In the Algebraic model, only the total mass is known and the biomass is calculated using an algebraic method. Model application in a loblolly pine plantation showed that mean monthly EMM production, mortality and decomposition estimates among three time periods ranged from 10.1 to 16.0 kg ha^{-1}, 6.6–15.0 kg ha^{-1}, and 1.4–6.1 kg ha^{-1}, respectively, when using the Biomarker model, while these estimates ranged from 24.8 to 35.7 kg ha^{-1}, 15.5–22.8 kg ha^{-1}, and 5.7–9.8 kg ha^{-1}, respectively, when using the Algebraic model, demonstrating the importance of assessing temporal changes. Model validation indicated that EMM estimates were more reliable for short-term compared to long-term incubation (184 vs. 322 days). Our method could improve EMM estimation by accurately assessing temporal changes in EMM production, mortality and decomposition in forests.

1. Introduction

In forest ecosystems, fine roots often form symbiotic associations with mycorrhizal fungi to increase nutrient acquisition via extensive networks of extramatrical mycorrhizal mycelia that proliferate throughout the soil, effectively increasing root system absorptive area. In return, the mycorrhizal fungi receive a significant allocation of carbohydrates from the host plants (Hobbie, 2006). Ectomycorrhizal fungi are a major mycorrhizal type and play an important role in soil organic matter formation and soil carbon (C) cycling in temperate and boreal forests (Cairney, 2012; Drigo et al., 2012; Clemmensen et al., 2013). Production of extramatrical mycelia of ectomycorrhizal fungi (EMM) in temperate and boreal forests has been estimated to range from 50 to 2700 kg ha^{-1} yr^{-1}, with an average of approximately 260 kg ha^{-1} yr^{-1} (Ekblad et al., 2013, 2016; Hendricks et al., 2016; Hagenbo et al., 2017). At the ecosystem scale, EMM production has been estimated to account for 27% of net primary production (NPP) in a mixed conifer-deciduous forest in California (Allen and Kitajima, 2014), and around 6% of NPP in boreal forests and loblolly pine plantation forests (Ekblad et al., 2013, 2016). However, unquantified uncertainty exists in EMM estimates mainly due to methodological limitations (Wallander et al., 2013). EMM extend longer distance than absorptive fine roots in soils, and dead EMM can not be visibly distinguished from living EMM (Wallander et al., 2013). EMM biomass and total mass (sum of EMM necromass and biomass) are usually assessed using chemical markers as proxies (Wallander et al., 2001). Differences between EMM and fine roots limit the application of methods used in fine root studies (e.g. minirhizotron, ingrowth core and soil coring) to estimating EMM production. For example, the dynamics of diffuse mycelia can not be well captured by the minirhizotrons (Allen and Kitajima, 2014). In addition, estimating actual EMM production using minirhizotrons requires conversion of EMM length and diameter data to biomass per unit soil volume. However, the only published conversion factor was determined in laboratory culture, failing to reflect variable field conditions (Van Veen and Paul, 1979). The ingrowth bag/core method can give a direct estimate of EMM mass, and therefore overcome some of the drawbacks associated with the minirhizotrons (Wallander et al., 2001; Parrent and Vilgalys, 2007; Hendricks et al., 2016; Hagenbo et al., 2017). However,

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it has two disadvantages. First, small bags/cores could have higher EMM density than large bags/cores at the initial assessment stage. Further, both sizes may have uniform density when EMM reach a new equilibrium state, but this represents the net standing mass rather than EMM production. Second, EMM decomposition has been ignored in most ingrowth bag/core studies (Hagerberg et al., 2003; Nilsson and Wallander, 2003; Parrent and Vilgalys, 2007; Hendricks et al., 2016), which potentially leads to significant underestimation of EMM production because some EMM have quite fast decomposition rates (Koide and Malcolm, 2009; Fernandez and Koide, 2012, 2014). To overcome the inherent disadvantages in the conventional ingrowth bag/core methods, Ekblad et al. (2016) proposed a new model to estimate EMM production, mortality (e.g. biomass turnover) and decomposition (e.g. necromass turnover) by assuming a constant biomass and necromass turnover rates. However, this assumption ignores the evident temporal changes in EMM production and mortality in forests Allen and Kitajima, 2013, reducing the reliability of estimates. In addition, the estimation of all EMM dynamics parameters was performed using Bayesian principles without verifying underlying probability distributions, which may result in unquantified error in EMM estimates (Ekblad et al., 2016).

In this study, we modified the conventional ingrowth bag/core method by adding a set of EMM decomposition experiments. Two models, the Biomarker model and the Algebraic model, were developed to assess the temporal changes in EMM production, mortality and decomposition by integrating EMM decomposition dynamics with the ingrowth bag/core data. In the Biomarker model, EMM biomass and total mass (sum of EMM necromass and biomass) are input parameters and are determined by using biomarkers as proxies. In the Algebraic model, EMM total mass value is an input parameter and is determined directly by measuring the weight loss in sand-filled bags/cores incubated under field conditions.

2. Materials and methods

2.1. Model description

In the Biomarker model, ergosterol or phospholipid fatty acid 18:2 w 6, 9 (PLFA) is used as the biomarker for EMM biomass (Wallander et al., 2001, 2013; Olsson et al., 2003), while chitin is adopted as the biomarker for EMM total mass (Ekblad et al., 1998, 2016). The necromass is estimated as the difference between the converted biomass and the total mass values. In the Algebraic model, the total mass is determined using a combustion method and the necromass is calculated. For both models, the EMM mass loss pattern is assessed using the litterbag method (Fernandez and Koide, 2012). The decomposing materials used to quantify EMM decomposition rates are obtained by harvesting the EMM growing into sand-filled ingrowth bags/cores.

The sampling frequency is limited to 4 times per year, as higher sampling frequency results in greater error propagation in EMM estimates. This is a compromise between practicality and accuracy, as there is no way to maximize both (Berhongaray et al., 2013). The installation and sampling regimes of ingrowth bags/cores and EMM litter-bags used are shown in Fig. 1 and are described later in the text. All variables and parameters are listed in Table 1.

2.1.1. Biomarker model

The incubation period is divided into 4 intervals in a year based on EMM seasonal dynamics; T_i is the time length of interval i ($1 \le i \le 4$) (Fig. 2 a, b). The biomass (B_i) and necromass (N_i) at the end of any given interval i are

$$B_i = \sum_{j=1}^{i} B_{i-(j)} \tag{1-1}$$

$$N_i = \sum_{j=1}^{i} N_{i-(j)} \tag{1-2}$$

where $B_{i-(j)}$ is the biomass remaining at the end of interval i from that produced in interval j, while $N_{i-(j)}$ is the necromass remaining at the end of interval i from that dying in interval j ($1 \le j \le i \le 4$) (Fig. 2a and b).

Supposing time t is any given length in interval i ($1 \le i \le 4$), the production ($g_i(t)$), mortality ($m_i(t)$) and decomposition ($d_i(t)$) from the start of interval i to any given time (t) in interval i can be calculated by the following equations,

$$g_i(t) = B_i(t) - B_{i-1} + m_i(t) \tag{1-3}$$

$$m_i(t) = N_{i-(i)}(t) + d_{i-(i)}(t) \tag{1-4}$$

where $d_{i-(i)}(t)$ is the decomposition incurred from the start of interval i to time t of interval i due to EMM that died during interval i(Fig. 2b), where $N_{i-(i)}(t)$ is the necromass remaining of EMM that died in interval i at time t of interval i (Fig. 2b).

The mass loss pattern of EMM that died in interval i is determined by the litterbag method ($1 \le i \le 4$). The decomposition experiment is conducted at the start of interval i till the end of the ingrowth experiment, termed as DE i (Fig. 1).

EMM decomposition rate decreased over time (Fernandez and Koide, 2012, 2014), thus

$$\frac{dy(t)}{y(t)} = \lambda e^{-k\,t}\,dt \tag{1-5}$$

where $y(t)$ is the amount of EMM at time t, $\lambda e^{-k\,t}$ is the decomposition rate, λ and k are parameters. $y(t)$ can be solved according to the same procedure described in Li et al. (2013).

$$y(t) = y_0 e^{(-\lambda/k)(1-e^{-k\,t})} \tag{1-6}$$

where y_o is the initial mass. λ and k can be calculated based on EMM mass remaining in litterbags.

Taking the derivative of Eq. (1-4), then

$$\frac{dN_{i-(i)}(t)}{dt} = \frac{dm_i(t)}{dt} - \frac{dd_{i-(i)}(t)}{dt} \tag{1-7}$$

To quantify the mortality, EMM in interval i are assumed to die at a constant mortality rate (μ_i) and follow the same decomposition dynamics as those in litterbags in DE i. Then $\frac{dm_i(t)}{dt} = \mu_i$ and $\frac{dd_{i-(i)}(t)}{dt} = \lambda_i e^{-k_i\,t} N_{i-(i)}(t)$ when substituting $y(t)$ with $N_{i-(i)}(t)$ in Eq. (1-7).

$$\frac{dN_{i-(i)}(t)}{dt} = \mu_i - \lambda_i \cdot e^{-k_i\,t} \cdot N_i - (i)\cdot(t) \tag{1-8}$$

This linear first-order differential equation with variables $N_{i-(i)}(t)$ and t can be solved using standard procedures (Kreyszig, 1972).

The solution can be regrouped to obtain the following analytical expression for the mortality rate:

$$\mu_i = N_{i-(i)}(t)\cdot\alpha_{(t)} \tag{1-9}$$

$$\alpha_{(t)} = k_i \frac{e^{-(\lambda_i/k_i)e^{-k_i t}}}{E_1((\lambda_i/k_i)e^{-k_i t}) - E_1(\lambda_i/k_i)}$$

where $E_1(z) = \int_z^{\infty} \frac{e^{-x}}{x} dx$ is an exponential integral function (Abramowitz and Stegun, 1964; ch. 6).

$N_{i-(i)}(t) = N_{i-(i)}$ when $t = Ti$.

According to Eq. (1-2) and Fig. 2b, $N_{i-(i)}$ can be expressed as ($1 \le j \le i \le 4$)

$$N_{i-(i)} = N_i - \sum_{j=1}^{i-1} N_{i-(j)} \tag{1-10}$$