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Title	A new approach to estimate fine root production, mortality, and decomposition using litter bag experiments and soil core techniques
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#### A new approach to estimate fine root 1

production, mortality, and decomposition 2

#### using litter bag experiments and soil core 3

#### techniques 4

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6 Division of Forest and Biomaterials Sciences, Graduate School of Agriculture, 7 Kyoto University, Kyoto, Japan 606-8502 8 Phone: +81-75-753-6095 9 Fax: +81-75-753-6098 10 E-mail: aosawa@kais.kyoto-u.ac.jp 11 12 13 14 Abstract. 15 Aims: A new approach is proposed to estimate fine root production, mortality, and decomposition 16 that occur simultaneously in terrestrial ecosystems utilizing sequential soil core sampling or 17 ingrowth core techniques. 18 19 Methods: The calculation assumes knowledge of the decomposition rate of dead fine roots during 20 a given time period from a litter bag experiment. A mass balance model of organic matter 21 derived from live fine roots is applied with an assumption about fine root mortality and 22 decomposition to estimate decomposed dead fine roots from variables that can be quantified. 23 24 Results: Comparison of the estimated fine root dynamics with the decision matrix method and 25 three new methods (forward estimate, continuous inflow estimate, and backward estimate) in a ca. 26 80-year-old *Chamaecyparis obtusa* plantation in central Japan showed that the decision matrix 27 nearly always underestimated production, mortality, and decomposition by underscoring the 28 values of the forward estimate, which theoretically underestimates the true value. The fine root 29 production and mortality obtained by the decision matrix were on average 14% and 38% lower 30 than those calculated by the continuous inflow estimate method. In addition, the values by the 31 continuous inflow estimate method were always between those calculated by the forward estimate 32 and backward estimate methods. The latter is known to overestimate the true value. 33 34 Conclusions: Therefore, we consider that the continuous inflow estimate method provides the best 35 estimates of fine root production, mortality, and decomposition among the four approaches 36

37

compared.

38 Key words: continuous inflow estimate, decision matrix, decomposition, fine

39 roots, litter bag, mortality, production

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## 41 Introduction

42 Terrestrial ecosystems interact with the atmosphere by affecting its carbon dioxide 43 concentration (Fung et al. 1983; Heimann et al. 1989). Therefore, good quantitative 44 knowledge of terrestrial carbon dynamics is necessary to understand potential changes in 45 the earth's climate system (Cox et al. 2000; Melillo et al. 2002; Heimann and Reichstein 46 2008). Translocation of assimilates to belowground organs of plants in terrestrial 47 ecosystems in order to grow fine roots and to support mycorrhizae is an important part of 48 net primary production (NPP). In fact, the proportion of total annual NPP allocated to 49 the belowground production may be more than 50% in some forests (Vogt et al. 1996) 50 and even more in boreal ecosystems in cold climates (Osawa et al. 2010). However, the 51 contribution of fine roots has not been fully incorporated into the estimates of NPP in 52 natural ecosystems (Hendrick and Pregitzer 1993; Lopez et al. 2001) because of the 53 difficulty of accurately measuring its pools and fluxes using available tools and 54 approaches.

55 Various methods have been developed for estimating the dynamics of fine roots in 56 natural ecosystems. Major methods include sequential soil core (Persson 1980; Ostonen 57 et al. 2005), ingrowth core (Finér et al. 1997; Ostonen et al. 2005), minirhizotron 58 (Hendrick and Pregitzer 1993; Majdi and Őhrvik 2004), nitrogen budget (Nadelhoffer et 59 al. 1985), and ecosystem carbon balance (Ågren et al. 1980), among others. Sequential 60 soil core and ingrowth core methods utilize several different approaches to calculate 61 production, mortality, and decomposition using changes in the pools of live and/or dead 62 fine roots (Vogt et al. 1998; Hendricks et al. 2006). Several in-depth comparisons (Neill 63 1992; Majdi 1996; Hertel and Leuschner 2002; Ostonen et al. 2005; Hendricks et al. 64 2006) and reviews (Vogt et al. 1996, 1998; Gill and Jackson 2000; Majdi et al. 2005; 65 Noguchi et al. 2007) of these methods have been published. Despite these publications, 66 there is still no consensus among belowground researchers on what methods most 67 realistically reflect changing carbon pools in the belowground part of the ecosystem. 68 Currently, root scientists generally agree that simultaneous fine root growth and 69 mortality cannot be measured directly with the sequential soil core and ingrowth core 70 methods (Kurz and Kimmins 1987; Santantonio and Grace 1987; Majdi 1996; Majdi et al. 71 2005). The minirhizotron technique could allow measurement of production and 72 mortality simultaneously (Hendrick and Pregitzer 1993; Steele et al. 1997; Ruess et al. 73 2003) but it has limitations (Majdi 1996; Dannoura et al. 2008). A method of

74 calculating fine root dynamics with the decision matrix was proposed for sequential soil 75 core and ingrowth core methods (McClaugherty et al. 1982; Fairley and Alexander 1985). 76 Various versions exist and have been widely used (e.g. Hertel and Leuschner 2002; 77 Ostonen et al. 2005; Hishi and Takeda 2005; Kaye et al. 2005; Hendricks et al. 2006; 78 Konôpka et al. 2006, 2007; Jourdan et al. 2008; Xiao et al. 2008; Hertel et al. 2009; 79 Jiménez et al. 2009; Murach et al. 2009; Mei et al. 2010; Yang et al. 2010). The 80 decision matrix could estimate the changing values of fine root production, mortality, and 81 decomposition, but it has a serious disadvantage. The problem is summarized in the 82 following discussion. 83 The decision matrix provides a method to estimate fine root production, mortality, 84 and decomposition during a given period, as illustrated in Table 1 (Fairley and Alexander 85 1985). For example, if  $\Delta B$  and  $\Delta N$  are defined as changes in fine root biomass and 86 necromass between two observations, and if both of these quantities have positive values, 87 fine root production (g) during this period is  $\Delta B + \Delta N$ . Mortality (m) is  $\Delta N$ . Note that 88 decomposition (d) was assumed as zero. In contrast, if  $\Delta B \ge 0$ , but  $\Delta N < 0$ , then  $g = \Delta B$ 89 and  $d = -\Delta N$ , under an assumption of m = 0. Values of g, m, and d for other 90 combinations of  $\Delta B$  and  $\Delta N$  values are calculated accordingly (Table 1). The decision 91 matrix has two notable characteristics. First, fine root production (g), mortality (m), and 92 decomposition (d) are calculated from knowledge of the changes in the pools of fine root 93 biomass ( $\Delta B$ ) and necromass ( $\Delta N$ ) but without knowledge of decomposition (d) or its 94 rate. Second, some assumptions used for the calculation of g, m, and d are intuitively 95 understandable; specifically that d = 0 for a condition of  $\Delta B \ge 0$  and  $\Delta N \ge 0$ . However, it 96 is also clear that decomposition may or may not be zero in this case. Assumptions for 97 other conditions are also neither immediately clear nor acceptable. In reality, the mass 98 balance of  $\Delta B$  and  $\Delta N$  with mass flux of g, m, and d should lead to a relationship:  $g = \Delta B$ 99  $+\Delta N + d$  and  $m = \Delta N + d$  (explained fully in the next section) with non-negative values 100 of g, m, and d. Therefore, the decision matrix only provides underestimates of the 101 variables of interest because production, mortality, or decomposition must be ignored in 102 specific calculations (Fairley and Alexander 1985). The discrepancy between the true 103 value and the calculation by the decision matrix is unknown due to lack of information 104 about concurrently occurring decomposition. Therefore, a more coherent approach is 105 desirable. 106 Santantonio and Grace (1987) proposed a modeling approach where the fine root 107 dynamics were evaluated by mass balance equations similar to those assumed in the 108 development of the decision matrix (McClaugherty et al. 1982; Fairley and Alexander

109 1985). The advantage of Santantonio and Grace's (1987) approach is the evaluation of

110 mortality and decomposition through modeling of the decay coefficient for dead fine

111 roots as a function of soil temperature, temperature coefficient, and reference rate of fine

112 root decomposition (their Equation 11). However, this approach (Santantonio and Grace 113 1987) has not been readily utilized by researchers because it demanded additional 114 measurement, estimation, and modeling of variables other than the decay coefficient itself 115 (Vogt et al. 1998). 116 The purpose of the present study is to propose a coherent and more practical approach 117 for estimating fine root production, mortality, and decomposition that occur 118 simultaneously. First, the fine root mass balance equations are solved mathematically 119

with acceptable assumptions for the instantaneous rates of fine root mortality and

120 decomposition. Then, fine root production, mortality, and decomposition are estimated 121 from the changes in fine root biomass and necromass, and the decomposition ratio of 122 dead fine roots during a measurement interval. The first two variables are provided by 123 either sequential soil core measurements or ingrowth core experiments. An estimate of 124 the third variable (decomposition ratio of dead fine roots) is also necessary. We show 125 that the complex modeling of the decay coefficient in Santantonio and Grace's (1987) 126 method can be replaced by a simple fine root litter bag experiment. In the estimation of 127 fine root mortality and decomposition with ordinary soil core techniques, none of the 128 methods, except for Santantonio and Grace's (1987) approach could take into account 129 simultaneous changes of these variables during a sampling interval. The present study 130 proposes a new equation that can be used to calculate these variables. We will compare 131 the estimated values between the decision matrix and the new methods that we describe. 132 In this study, fine root production is defined as the total amount of fine root ingrowth 133 during a specified period. This is conceptually similar to NPP of aboveground organs 134 and is approximately equivalent to what is often referred to as "belowground NPP". 135

#### A mass balance model and rational for field 136

#### 137 measurement

138 Santantonio and Grace's model (1987) suggests that if the sequential soil core or 139 ingrowth core method is combined with an additional field experiment to assess fine root 140 decomposition, we could estimate fine root production, mortality, and decomposition that 141 occur simultaneously in a given ecosystem. As in Santantonio and Grace (1987), we 142 assume a simple mass balance model with compartments and flow of organic matter of 143 fine root origin during a discrete time interval. We then estimate the flow and change in 144 stock of organic matter during this interval. 145 Let us first consider a volume of soil in a natural ecosystem consisting of mineral soil, 146 fine roots, and organic matter derived entirely from fine roots. The organic matter

147 originating from aboveground organs is ignored. This volume of soil contains a certain

- 148 amount of live fine roots at time i (denoted as  $B_i$ ) and dead organic matter of fine root
- 149 origin  $(N_i)$ . Hereafter, the former is called fine root biomass and the latter fine root
- 150 necromass. The fine root biomass and necromass change at time j (where j > i) to  $B_j$  and
- 151  $N_j$  due to new growth of fine roots into the soil volume, their mortality, and their
- 152 decomposition. If  $g_{ij}$  and  $m_{ij}$  denote fine root production and mortality into or within the
- 153 soil volume between the times i and j (Fig. 1), the change in fine root biomass ( $\Delta B$ )
- 154 satisfies the following mass balance equation:  $\Delta B = B_i B_i = g_{ii} m_{ij}$ . Similarly, the
- 155 change in fine root necromass ( $\Delta N$ ; Fig. 1) satisfies the following:  $\Delta N =$
- 156  $N_i N_i = m_{ii} d_{ii}$ , where  $d_{ij}$  is decomposition of fine root necromass between i and j.

157 Therefore, these mass balance equations suggest that the fine root production and 158 mortality are calculated as,

- 159  $g_{ii} = \Delta B + \Delta N + d_{ii} \tag{1}$
- $160 mtextbf{m}_{ii} = \Delta N + d_{ii} (2)$

161 where all terms have the dimension g m<sup>-2</sup>  $\Delta t$  d<sup>-1</sup> and  $\Delta t = j - i$  in the following 162 discussion. These relationships can be applied with periodic soil sampling and a root 163 litter bag experiment to estimate fine root production, mortality, and decomposition of a 164 given site simultaneously.

165

#### 166 **Periodic soil sampling**

167 Soil cores of a specified diameter and volume are collected at different times, either by 168 harvesting natural soils or by recovering fine root ingrowth cores that were installed at the 169 beginning of the experiment at time 0. The first set of cores is collected at time i (0 < i;170 Fig. 2) for measurement of fine root biomass and necromass in the soil volume. The 171 second set is collected later at time j (i < j; Fig. 2). The interval between the two 172 collections is generally several weeks to a few months. Fine root biomass and 173 necromass are also measured in the second set of soil cores. Here we assume that the 174 two sets of cores sampled at times i and j have the same initial condition and are left 175 under the same environment. Consequently, the patterns of fine root production, 176 mortality, and decomposition are considered identical between them. In other words, we 177 assume that fine root biomass and necromass at time i will be the same for the two sets. 178 In reality, there will be variations in the observed values among the cores of the same set. 179 However, we assume that the mean values are the same for the same variable at the same 180 time of observation when the growing conditions are identical. 181

### 182 **Root litter bag experiment**

183 Litter bags are used to evaluate the decomposition ratio of dead fine roots. The fine root 184 litter bags containing a known amount of dead fine roots are set in the soil horizon at time i. The dead fine roots are denoted as  $N_i^{C}$ , where the superscript C represents a value 185 186 obtained from the litter bags (See Litter bag C in Fig. 2). The litter bags are recovered at 187 time j (Fig. 2) for evaluating the decomposition ratio of fine root necromass. As there 188 were no live fine roots in the litter bag when they were set in the soil, we also have condition  $B_i^{C} = 0$ . If fine root production between times i and j is zero, by artificially 189 190 excluding fine root growth into the litter bag, the fine root biomass at time j will also be zero. Therefore, Equation 1 reduces to  $d_{ij}^{\ C} = N_i^{\ C} - N_j^{\ C}$  from which decomposition 191 of fine root necromass can be estimated. Where  $\gamma_{ii}$  is the decomposition ratio 192 (dimensionless) of fine root necromass between times i and j, then 193  $\gamma_{ij} = d_{ij}^{\ C} / N_i^{\ C} = 1 - N_i^{\ C} / N_i^{\ C}$ 194 (3). 195 Decomposition of naturally dead fine roots and artificially severed live fine root segments 196 may represent different phenomena (Publicover and Vogt 1993). However, severed 197 roots are often used in decomposition experiments, as is the case in the present study. 198 Publicover and Vogt (1993) discuss possible variations and mechanisms of

- 199 decomposition in roots of varying origins.
- 200

#### 201 Calculating decomposition of dead fine roots

A realistic estimate of fine root decomposition between times i and j may be obtained by assuming that the fine roots die continuously at a constant instantaneous mortality of  $\sigma$ (g m<sup>-2</sup> d<sup>-1</sup>) and disappear at an instantaneous decomposition of  $\gamma \cdot N$  (g m<sup>-2</sup> d<sup>-1</sup>) with a constant decomposition rate  $\gamma$  (d<sup>-1</sup>), where  $\sigma$  and  $\gamma$  are formally defined as

206 
$$m_{ij} = \int_{i}^{j} \sigma dt$$
 and  $d_{ij} = \int_{i}^{j} \gamma \cdot N dt$ , and N is fine root necromass at time t (i  $\leq t \leq j$ ).

- 207 These assumptions are identical to those used by Santantonio and Grace (1987). The
- 208 assumption of constant root mortality,  $\sigma$ , must be introduced due to lack of sufficient
- 209 knowledge about its behavior but it can vary for different observation intervals. Given
- 210 these assumptions, the instantaneous necromass mass balance can be expressed as
- 211  $dN/dt = -\gamma \cdot N + \sigma$ . This is a linear first-order differential equation (Kreyszig 1972),
- and solving for N yields  $N = \sigma / \gamma + (N_i \sigma / \gamma) \cdot e^{-\gamma \cdot (t-i)}$ . By applying this

213 relationship to the present situation, we obtain an expression for fine root decomposition

- 214 between times i and j as (see Appendix for derivation)
- 215  $d_{ij} = -\Delta N (\Delta N / \gamma_{ij} + N_i) \cdot \ln(1 \gamma_{ij})$ (4),

where,  $\gamma_{ij}$  is as defined in Equation 3. Therefore, the amount of the decomposed dead fine roots can be estimated from three variables ( $\Delta N$ ,  $N_i$ , and  $\gamma_{ij}$ ) that can be measured by soil sampling and a relatively simple experiment. The modeling exercise (Santantonio and Grace 1987) is no longer required. The estimate of  $d_{ij}$  from Equation 4 may be referred to as the continuous inflow estimate. Comparison of Equations 2 and 4 yields

222 
$$m_{ij} = -\left(\Delta N / \gamma_{ij} + N_i\right) \cdot \ln(1 - \gamma_{ij})$$
(5).

223 An estimate of fine root mortality ( $\sigma$ ), which was assumed to be constant during a 224 measurement period can be calculated as  $m_{ij}/\Delta t$ . Note that mortality is expressed neither 225 by  $\Delta N$  nor  $\Delta B$  alone as in the decision matrix. In this new approach, the decomposition 226 ratio ( $\gamma_{ij}$ ) is also related to mortality.

227 Decomposition of fine root necromass between times i and j  $(d_{ij})$  can also be 228 approximated as the estimated decomposition ratio multiplied by the fine root necromass 229 at time i

230  $d_{ij} = \gamma_{ij} \cdot N_i$  (6).

This expression (forward estimate) assumes that decomposition occurring between times i and j originates from the necromass present at time i and that there is neither fine root mortality nor associated decomposition of the newly dead fine roots between i and j (in order to calculate the amount of decomposition). Therefore, the decomposition estimated with Equation 6 is an underestimate.

Alternatively, if  $N_j$ , necromass at time j through decomposition of dead fine roots between times i and j with a decomposition ratio of  $\gamma_{ij}$ , is known, but  $N_i$ , necromass at time i, is not known, fine root decomposition can also be estimated (backward estimate) as

240  $d_{ij} = \gamma_{ij} \cdot N_j / (1 - \gamma_{ij})$ (7).

This representation means that new dead fine roots may be added between times i and j, all of which will occur as a pulse immediately after time i. However, in reality, fine root mortality can occur anytime between i and j, which makes the residence time of these newly dead fine roots shorter than j–i. Therefore, the decomposition estimate from Equation 7 will be an overestimate. These considerations suggest that the true value of fine root decomposition lies between the values calculated by Equations 6 and 7 and the values will coincide only when there is no fine root mortality between times i and j. 248 Therefore, the estimate of  $d_{ij}$  from Equation 4 may be considered the most realistic 249 theoretical value among the values calculated by Equations 4, 6, and 7. 250 Fine root production  $(g_{ii})$  and mortality  $(m_{ii})$  are then calculated by Equations 1 251 and 2. Estimation using these relationships is practical. It is also expected to be 252 applicable in many types of terrestrial ecosystems for varying amounts of production, 253 mortality, and decomposition. The ability of the present approach to detect changes in 254 fine root biomass and necromass pools is the same as that of the methods proposed earlier 255 (e.g., decision matrix). However, the treatment of decomposition is different. The 256 present approach takes into account decomposition directly, whereas the decision matrix 257 in effect ignores decomposition, leading to underestimation when decomposition is not 258 negligible.

259

## 260 Materials and methods

### 261 Study site

262 The proposed method of estimating fine root production, mortality, and decomposition 263 was applied to a ca. 80-year-old plantation of *Chamaecyparis obtusa* Endl. (Hinoki 264 cypress) at Ryukoku Forest, Ohtsu, Japan, at 34°58'N, 135°56'E. The elevation of the 265 stand is 130 m above sea level. The soil of the study area is characterized by sand and 266 small round gravel stones, and is classified as yellowish brown forest soil derived from 267 lacustrine sediments of Cenozoic origin belonging to the Kobiwako Group (Ministry of 268 Land, Infrastructure, Transportation and Tourism 1982). The Ryukoku Forest is a 269 secondary forest of mainly canopy trees of *Quercus serrata* Thunb. and *Pinus densiflora* 270 Sieb. et Zucc. that used to be managed communally. The forest supplied agricultural 271 fertilizer and wood material for local farmers, but now the area is abandoned. The C. 272 obtusa stand has not been managed for at least 40 years (Miyaura 2009) and shows signs of active self-thinning (Osawa and Allen 1993). The stand is about  $800 \text{ m}^2$  with stem 273 274 density, mean stem diameter at breast height, canopy tree height, and basal area of C. 275 *obtusa* at 1175 ha<sup>-1</sup>, 22 cm, 20 m, and 51 m<sup>2</sup>/ha, respectively (Tago J *unpublished data*). 276 In addition, the stand also has some canopy and subcanopy trees of *Q. serrata*, *P.* 277 densiflora, and Ilex pedunculosa Miq.. 278

### 279 Ingrowth core experiment in 2007

280 To assess fine root dynamics, a cylindrical tube 32 mm in diameter and 21 cm in length 281 was used as the fine root ingrowth core and the root litter bag. The cylindrical tube is 282 made of plastic mesh with approximately 3 mm mesh openings and a mesh bottom made 283 of the same material as the cylinder wall. This cylinder was filled with soil from the 284 study site, but all live roots, dead roots, and decomposing organic matter (diameter  $\phi$  > 285 0.5 mm) were removed by washing the soil ten times with tap water in a container and 286 sieving the supernatant fluid with suspended organic matter through a 0.5 mm mesh sieve 287 after each washing. Fine particles ( $\phi \leq 0.5$  mm) of mineral soil and particulate 288 organic matter in the supernatant fluid were saved, dried at room temperature, broken into 289 shatters, and mixed with the soil sediment in the container for use in the experiment. 290 Some of the soil-filled plastic mesh cylinders were covered with a 'root-impermeable water-permeable (RIWP) sheet<sup>®</sup>' (Toyobo Co., Osaka, Japan) to exclude fine roots that 291 292 could grow into the cylinder. The RIWP sheet has a pore size of approximately  $6\mu m$ 293 and blocked practically all ingrowth of fine roots; however, fine particles of soil, rain 294 water, and other microorganisms could penetrate through the sheet.

295 A known mass of dead fine roots of C. obtusa was added to each of the RIWP sheet-296 covered cylinders so that they acted as root litter bags. Severed live fine root segments 297 were collected, dried at room temperature, and used as dead fine roots in the litter bags in 298 this experiment (see Publicover and Vogt (1993) for caution). Soil was included in the 299 litter bags to improve the physical contact of the soil particles with the inserted dead 300 roots; however, use of ordinary litter bags without soil is a possible alternative. In total, 301 60 soil cylinders were prepared; 30 of which were covered with the RIWP sheet. The 302 tops of all cylinders were covered with thin translucent fabric to exclude input of 303 aboveground litter.

304 Six sampling lines, approximately 30 m long, were established in the stand. They 305 were parallel to one another and about two-meters apart. The ground surface along the 306 sampling lines was marked at one-meter intervals to indicate locations for the soil 307 cylinders. The sampling points for the 30 soil cylinders (fine root ingrowth cores) and 308 the 30 root litter bag cores were selected randomly. The 30 ingrowth cores were buried 309 singly in early July 2007 after coring the ground with a stainless steel tube 38 mm in 310 diameter and 45 cm in length by pounding it 20 cm into the ground. The narrow space 311 between the ingrowth core and the inner wall of the cored hole was filled with the same 312 soil as that used to fill the ingrowth core. 313

In mid-August 2007, six ingrowth cores were chosen randomly, taken out of the ground by creating 20-cm deep incisions around the core with a knife and the ingrowth 315 core was pulled out by hand. These six ingrowth cores correspond to Core A in Fig. 2. 316 At the same time, six root litter bag cores were established at randomly selected locations 317 among the remaining sampling points (corresponding to Litter bag C in Fig. 2). Six 318 weeks later, these six litter bag cores and another set of six randomly selected regular 319 ingrowth cores (Core B in Fig. 2) were recovered on the same day by a method similar to 320 the one described above. The collected soil cylinders were brought to the laboratory. 321 The contained soil was washed using the same method applied in preparation of the soil 322 material. Then the recovered organic material was sorted into live fine roots, dead fine 323 roots, and other organic matter. The live and dead fine roots were differentiated by their 324 color and resilience (Hishi and Takeda 2005; Konôpka et al. 2006) because more exact 325 methods were not practical in field studies. The roots of different tree species were not 326 distinguished in the present study; however, most were from C. obtusa. They were 327 dried in a forced air oven at 80°C for 48 hours, and biomass and necromass were 328 measured separately. The mass of the live fine roots was considered fine root biomass, 329 and the mass of the dead fine roots combined with other organic matter ( $\phi > 0.5$  mm) 330 was regarded as necromass.

331 Each time the soil cylinders corresponding to Core B and Litter bag C were 332 recovered, another set of six root litter bag cores was buried at randomly selected 333 sampling points. They were taken out of the ground on the next sampling occasion (new 334 Litter bag C; generally six weeks later) along with a set of six randomly selected fine root 335 ingrowth cores (new Core B). Core B from the previous sampling time becomes the 336 new Core A. Analysis of these cores gave the biomass and necromass values at the 337 experiment's next time step. By repeating this process, fine root production, mortality, 338 and decomposition were estimated until December 2007, generally at six-week intervals. 339 In addition, six root litter bags were set, using a method similar to that employed initially, 340 and were recovered in mid-August so that the fine root decomposition ratio during the 341 first period could also be estimated.

342

### 343 Sequential soil core sampling in 2010

Soil cores were sampled sequentially with the same stainless steel tube at randomly selected points, generally at nine-week intervals between June 22 and December 16, 2010. The potential sampling points were located at one-meter intervals along the sampling lines in the same *C. obtusa* stand. Usually three cores were collected at each sampling. Root litter bags of the same construction as the 2007 experiment were prepared and buried each time soil samples were collected. The root litter bag cores recovered at each sampling was generally three. The collected soil cylinders and 351 contents of the root litter bag cores were washed in tap water applying the same method 352 used in the 2007 experiment. Then, after oven-drying at 80°C for 48 hours, live fine 353 roots, dead fine roots, and other organic matter ( $\phi > 0.5$  mm) were quantified. Only 354 the mass of dead fine roots was regarded as necromass in the 2010 study because the 355 origin of decomposing organic matter was generally unknown. The stand was thinned 356 to reduce the tree density and stem volume of C. obtusa by 47% and 36%, respectively, in 357 March 2010 (Tago J personal communication) for a separate study of forest growth prior 358 to the start of the present soil core sampling, which commenced on June 22, 2010. 359

#### 360 Mass flux calculation and statistical analysis

361 The measured values of fine root biomass and necromass in each soil core, ingrowth core, 362 and root litter bag were divided by the area of the inner cross-section of the stainless steel pipe (0.00101 m<sup>2</sup> for soil core) or of the ingrowth core and root litter bag (0.000706 m<sup>2</sup>) 363 364 to obtain the mass per square meter of the ground. Then, they were divided by the duration of each experiment in days ( $\Delta t = i - i$ ) to calculate the mass flux in g m<sup>-2</sup> d<sup>-1</sup>. 365 366 The 95% confidence limits of the estimated means were calculated by the bootstrap 367 method with bias-corrected percentile approach by randomly sampling a set of mass data 368 n times with replacement (when sample size is n) to obtain the means, then repeating the 369 process 1,000 times to generate the 95% confidence interval (CI) (Efron 1979; Efron and 370 Gong 1983). Decomposition is calculated from a relatively complex equation (Equation 371 4), making use of traditional statistical inference inappropriate. Therefore, significant 372 differences in the means of biomass or necromass (Hendricks et al. 2006) were not 373 applied to calculate production, mortality, and decomposition in the present study, except 374 for comparisons in Table 2. 375 The following is an example of the mass flux calculation. Data collected on October 376 11 and November 10, 2007 (55 days apart) are used. The fine root necromass in the root 377 litter bag core was 0.739 g on October 11. On November 10, it was 0.482 g. 378 Therefore, the root decomposition ratio during this period ( $\gamma_{ii}$ ) was 0.347 (Equation 3). 379 The mean values of fine root biomass and necromass per core were 0.0101 g and 0.0259 380 g, respectively, on October 11. These values changed to 0.165 g and 0.0439 g, 381 respectively, on November 10. Therefore,  $\Delta B = 0.165 - 0.0101 = 0.154$  g and  $\Delta N =$ 382 0.0439-0.0259 = 0.0180 g per core. Fine root decomposition during this period will be 383 calculated by Equation 4 as  $d_{ij} = -0.018 - (0.018/0.347 + 0.0259)\ln(1-0.347) = 0.015$  (g 384 per core per 55 days). Then fine root mortality (m) and production (g) become m =385 0.018 + 0.015 = 0.033 and g = 0.154 + 0.018 + 0.015 = 0.187 (g per core per 55 days;

Equations 2 and 1). The results of calculation can be translated to d = 0.392, m = 0.855, and g = 4.849 (g m<sup>-2</sup> d<sup>-1</sup>).

388

### 389 **Results**

### 390 Ingrowth core experiment in 2007

391 Based on the continuous inflow estimate (Equation 4), the observed decomposition,  $d_{ii}$ , ranged between 0.14 and 0.76 g  $m^{-2} d^{-1}$  and showed little seasonal variation (black 392 pattern in Figs. 3a-3d). Values of fine root mortality,  $m_{ij}$ , ranged between 0.85 and 1.57 393 394  $g m^{-2} d^{-1}$ . The mortality was relatively low during summer and fall, and increased 395 during winter between November and December (black pattern in Figs. 3e-3h). 396 Seasonal dynamics of fine root production,  $g_{ij}$ , showed a different pattern. It was low in the summer (August value was 1.42 g m<sup>-2</sup> d<sup>-1</sup>), peaked in October at 4.84 g m<sup>-2</sup> d<sup>-1</sup>, and 397 398 became nearly zero through November and December in the continuous inflow model 399 (black pattern in Figs. 3i-3l). The bootstrap 95% CI of the estimated fine root 400 production was large, mostly due to the small sample size (generally six) and propagation 401 of errors associated with the measurement of  $m_{ij}$  and  $d_{ij}$  (see Equations 1, 2, 4, and 5). 402 Due to the large 95% CI, fine root production was not distinguishable from zero in 403 November and December (Figs. 3k and 3l). 404 The calculated values of fine root production, mortality, and decomposition by the 405 forward estimate (Equation 6) were lower than those estimated by the continuous inflow 406 assumption (Equation 4). On average, the values were 47% lower for decomposition 407 (Figs. 3a-3d), 15% lower for mortality (Figs. 3e-3h), and 7% lower for production (Figs. 408 3i-3l). On the other hand, the values calculated by the backward estimate (Equation 7) 409 were always higher than the continuous inflow estimate. They were higher on average 410 by 59% for decomposition (Figs. 3a-3d), 18% for mortality (Figs. 3e-3h), and 8% for 411 production (Figs. 3i-3l). The fine root production calculated by the forward estimate 412 and the continuous inflow assumption were negative during the fourth period (ending on 413 December 21; Fig. 31); however, because negative values are ecologically unacceptable, 414 we assigned zero to them. The reason for the negative production is the non-closure of 415 mass balance with all non-negative terms in Equation 1. 416 The estimates of fine root decomposition by the decision matrix were zero for the first 417 three periods until November 10 (Figs. 3a-3c). In contrast, the decomposition estimates 418 by the forward, continuous inflow, and backward models were generally positive. The 419 pattern differed in the fourth period (ending on December 21) where the decision matrix 420 decomposition estimate was greater than both the forward and continuous inflow

421 estimates (Fig. 3d). On average, fine root decomposition estimated by the decision 422 matrix was 69% lower than that calculated by the continuous inflow assumption. 423 Relationships among the mortality values calculated by the decision matrix and by the 424 forward, continuous inflow, and backward models (Figs. 3e-3h) are similar to those 425 observed for fine root decomposition. The mortality values estimated by the decision 426 matrix were on average 31% lower than those calculated by the continuous inflow 427 assumption. The decision matrix also returned the lowest fine root production values 428 when the four approaches were compared (Figs. 3i-3l). The decision matrix production 429 estimates were on average 18% lower than the continuous inflow estimates. 430

### 431 Sequential soil core sampling in 2010

Patterns of the calculated fine root decomposition, mortality, and production by the four
methods (decision matrix, forward estimate, continuous inflow, and backward estimate)
in the sequential soil core sampling for 2010 (Fig. 4) were generally similar to those
observed in the 2007 ingrowth core experiment (Fig. 3). The decision matrix gave the
lowest estimates. The values calculated by the continuous inflow model were also
between those predicted by the forward and backward estimates.

438 The continuous inflow model (Equation 4) returned decomposition values,  $d_{ii}$ , 439 ranging between 0.27 and 0.42 g m<sup> $^{-2}$ </sup> d<sup> $^{-1}$ </sup>. It showed little seasonal variation, as evidenced in the 2007 data (black pattern in Figs. 4a-4c). Fine root mortality,  $m_{ij}$ , 440 ranged between 0.38 and 1.60 g  $m^{-2} d^{-1}$ . The mortality was somewhat high in summer 441 442 but decreased after August (black pattern in Figs. 4d-4f). The greatest value of fine root mortality was observed during the first period (ending on August 24) at 1.60 g m<sup>-2</sup> d<sup>-1</sup> 443 (Fig. 4d). Seasonal dynamics of fine root production,  $g_{ii}$ , was relatively stable, and 444 varied only between 2.07 and 2.38 g m<sup>-2</sup> d<sup>-1</sup> (black pattern in Figs. 4g-4i). This may 445 446 reflect the low fine root production at Ryukoku Forest in 2010 (Hattori 2011). The 447 bootstrap 95% CI of the estimated fine root production was large—a pattern similar to the 448 observation in 2007 (Figs. 3i-31). 449 The fine root decomposition estimated by the decision matrix were all zero, but 450 other methods predicted positive values (Figs. 4a-4c). Mortality estimated by the 451 decision matrix were on average 47% lower than that calculated by the continuous inflow 452 assumption. The decision matrix also led to the lowest fine root production estimates 453 when the four approaches were compared (Figs. 4g-4i). The production estimates from

454 the decision matrix were on average 15% lower than the continuous inflow estimates.

### 456 **Discussion**

457 Some previous studies reported that simultaneously varying fine root production, 458 mortality, and decomposition cannot be measured directly with sequential soil core or 459 ingrowth core methods (Kurz and Kimmins 1987; Santantonio and Grace 1987; Majdi 460 1996; Maidi et al. 2005); however, such measurement is possible with the addition of 461 litter bags as we have described in the present study. 462 Our results showed that the decision matrix nearly always gave values lower than 463 those calculated by the forward estimate (Figs. 3 and 4), which in itself is an 464 underestimate. This was because increments of both fine root biomass and necromass 465 were positive at nearly all periods, which forced the calculation of fine root 466 decomposition by the decision matrix to zero (Fairley and Alexander 1985), although 467 there were appreciable amounts of decomposition according to the other methods. In 468 one case, the decision matrix estimates were greater than those of other methods (Figs. 3d 469 and 3h). However, this is an anomaly created by non-closure of mass balance in fine 470 root dynamics due to the measurement error. Theoretically, the calculations by the 471 decision matrix are nearly always underestimates, and consequently cannot be regarded 472 as the best method.

473 The forward estimate is also bound to be an underestimate, because it only takes into 474 account decomposition of dead fine roots present at the beginning of the period 475 considered. It ignores decomposition of new roots that died during the measurement 476 period. On the other hand, the backward estimate will be an overestimate because it 477 assumes that death of new roots during the period occurs at one time immediately 478 following the start of the period under consideration. Therefore, the true value of 479 decomposition must lie between the values calculated by the forward and backward 480 estimates. The continuous inflow estimate consistently satisfies this condition (Figs. 3 481 and 4).

482 Another characteristic of the present examples of continuous inflow estimates is that 483 the magnitude of 95% CI tends to be large for fine root production (Figs. 3 and 4). This 484 is mainly due to the propagation of measurement errors in the calculation which involves 485 several variables (Equations 1 and 4) each of which contain variations. Propagation of 486 measurement error is a problem that cannot be avoided when several measurements are 487 added to derive a production estimate. There are mainly two sources of error. One is 488 addition of increments in biomass and necromass and decomposition during a single 489 measurement period (Equation 1). Each of these terms has its own variation, which 490 must be added to obtain that of production. The other source of error comes from 491 addition of production values for different measurement periods. Use of only the 492 significant differences will reduce the propagated error by omitting variation in non493 significant data; however, it cannot be free of error propagation as long as addition of
494 terms is involved. A relatively large sample size will be necessary to improve accuracy
495 substantially and the necessary sample size is likely to vary depending on plant species
496 and site conditions.

497 However, if the purpose of the study is to assess the magnitude of NPP or the 498 contribution of fine root production in total NPP, a continuous inflow estimate will be 499 more desirable. The estimate of fine root production by the decision matrix (as 500 compared to continuous inflow model) was 14% lower on average, and 38% lower in an extreme case, in the present study of the C. obtusa stand in 2007 and 2010. Therefore, 501 502 use of the decision matrix is likely to lead to substantial underestimation of NPP of the 503 ecosystem. The degree of error was even greater for fine root mortality. When 504 calculated by the decision matrix fine root mortality was 38% lower on average than the 505 value estimated by the continuous inflow assumption. In an extreme case, the decision 506 matrix estimate was 80% lower.

507 The effect of the use of different methods of calculation on fine root production over a 508 longer measurement period is also of interest. When only the significant differences of 509 biomass and necromass between measurement periods ( $\Delta B$  and  $\Delta N$ ) were added, decision 510 matrix led to an estimate of 256 g  $m^{-2}$  166 $d^{-1}$  for the 2007 experiment. In contrast, the decision matrix suggested 333 g m<sup>-2</sup> 166d<sup>-1</sup> when all  $\Delta B$  and  $\Delta N$  values were used for 511 512 calculation regardless of their significance (Table 2). We also obtained production of  $304 \text{ g m}^{-2} 166d^{-1}$  when only the first and last sampling data were used to calculate 513 514  $\Delta B + \Delta N$  (Table 2). The last calculation is identical to the estimate by the decision 515 matrix using the same two observations (Table 2), and is clearly an underestimate since 516 decomposition is ignored (Equation 1). When we use the same observations, but take 517 into account decomposition calculated using the mean decomposition rate (the parameter 518  $\gamma$  in Equation A2) for all periods and the continuous inflow estimate, the fine root production (Equation 1) becomes  $392 \text{ g m}^{-2}$  per 166 d (Table 2). Use of only significant 519 520 differences has been the method to avoid overestimation; however, it resulted in substantial underestimation (256 g  $m^{-2}$  166 $d^{-1}$ ) when combined with the decision matrix. 521 This value was 15% smaller than the known underestimate of 304 g  $m^{-2}$  166 $d^{-1}$ . It was 522 also 34% smaller than the continuous inflow estimate (392 g  $m^{-2}$  166d<sup>-1</sup>) calculated with 523 524 the first and last sampling data (Table 2). 525 Patterns of the estimated production were similar for the 2010 experiment. Decision matrix with data from all periods resulted in underestimation of 493 g  $m^{-2}$  177 $d^{-1}$  since 526 527 this value was equal to the estimate of  $\Delta B + \Delta N$  calculated by using only the first and last 528 sampling data without decomposition (Table 2). Another characteristic of the 2010 data

529 is that the estimates calculated only with the significant differences resulted in unusually

low values. This was because all differences in fine root biomass and necromass werenot significantly different between the measurement periods.

These observations and results of Table 2 suggest that use of only the significant differences tends to substantially underestimate fine root production, particularly when the decision matrix was used for calculation. Forward estimate also led to values lower than the known underestimate of  $\Delta B + \Delta N$  in the majority of cases. On the other hand, the continuous inflow estimate suggested stable production values regardless of the number of measurement periods applied.

538 The decomposition rates of fine root necromass that we observed in the present study 539 are probably reasonable. The decomposition rates ( $\gamma$ ; evaluated with Equation A2) were  $0.0071\pm0.0014$  (d<sup>-1</sup>) for a series of ca. six-week-long experiments in 2007. They 540 are equivalent to annual rates of  $2.61\pm0.53$  (y<sup>-1</sup>; Table 3). These are also translated as 541 monthly values of the decomposition ratio ( $\gamma_{ii}$ ) at 0.19±0.03 (dimensionless), or annual 542 543 values of 0.87±0.07 (dimensionless; Table 3) for a hypothetical condition that assumes 544 the rate does not change for an entire year (e.g., Equation A2). Similarly, the 545 decomposition rates ( $\gamma$ ) for the 2010 sequential soil cores were 0.0035±0.0007 (d<sup>-1</sup>) for 546 ca. two-month observation periods. They are equivalent to annual rates of  $1.30\pm0.28$ (y<sup>-1</sup>). They correspond to monthly rates  $\gamma_{ij}$  of 0.10±0.01 (dimensionless), or annual 547 548 values of 0.65±0.05. Therefore, the decomposition rates of dead fine roots observed in 549 the present study are generally of similar magnitude to those assumed by Publicover and 550 Vogt (1993) in their simulation: monthly  $\gamma_{ij}$  of 0.15 or annual  $\gamma_{ij}$  of 0.85 (Table 3). 551 Comparison of annual decomposition rates ( $\gamma$ ) among various ecosystems, presented 552 in Table 3 indicates that the estimates for the C. obtusa forest in the present study are 553 somewhat higher than those reported for other ecosystems. It is also noted that the 554 various ranges of decomposition rates are similar regardless of the methods used for 555 estimation. For example, when the method used in the present study was applied, the 556 annual  $\gamma$  was 0.16 for an old jack pine stand in Canada. This value is nearly equal to 557 the global mean for a conifer forest when regular envelope-type litter bags are used 558 (Table 3). Therefore, variation in observed decomposition rates is likely to have 559 originated primarily from differences in the characteristics of ecosystems, and not from 560 the methods used for analysis. In the present study, the annual decomposition rates were 561 extrapolated from data from field experiments that lasted 30-91 days during non-winter 562 seasons. True annual decomposition rates will be lower than those reported. In summary, both sequential soil cores and ingrowth cores could be used with root 563 564 litter bag experiments to estimate fine root production, mortality, and decomposition 565 occurring simultaneously, and could provide those parameters with mass-based flux variables (e.g.,  $g m^{-2} d^{-1}$ ). Generalized relationships were derived that allow calculation 566

567 of production, mortality, and decomposition from changes between observations in fine 568 root biomass and necromass, and from the decomposition ratio of dead fine roots 569 (Equations 1, 2, and 4). The decomposition estimate calculated with the continuous 570 inflow assumption (Equation 4) is considered the best among the four approaches 571 compared because it may closely represent the reality of the process of fine root 572 mortality. Accordingly, the continuous inflow approach would also be the best choice 573 for calculating fine root mortality and production (Equations 1 and 2). A large sample 574 size is necessary to reduce the 95% CI of the estimated production by the continuous 575 inflow estimate; however, it would be theoretically more appropriate than the decision 576 matrix which could not realistically take into account decomposition. Development of 577 the continuous inflow approach is significant not necessarily because it suggests values 578 different from those calculated by the decision matrix, but because it is applicable to 579 general situations regardless of the magnitude of decomposition of dead roots, root 580 mortality, and production. The decision matrix will give theoretically realistic values 581 only for situations where decomposition, mortality, or production is negligible. The 582 ability of the continuous inflow approach to estimate fine root dynamics with a simple 583 litter bag experiment is also a significant improvement over the method of Santantonio 584 and Grace (1987), in which decomposition must be estimated through relatively complex 585 modeling with additional environmental data.

586

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593

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*Continuous inflow estimate of d<sub>ii</sub> (Equation 4):* First, let us consider the decomposition

754

755

757

## 756 Appendix

process of dead fine roots occurring in a root litter bag treated with the 'root-impermeable water-permeable (RIWP) sheet' (see Materials and methods). The amount *N* of dead fine root is assumed to decompose with instantaneous decomposition of  $\gamma \cdot N$  where  $\gamma$ is the decomposition rate. This process can be described by a differential equation,  $dN/dt = -\gamma \cdot N$ . With a boundary condition,  $N = N^{C}_{i}$  at t = i, this differential equation is solved as

764 
$$N = N^C_i \cdot e^{-\gamma \cdot (t-i)}$$
(A1).

765 If  $N = N^{C}{}_{j}$  at t = j, and  $\Delta t = j - i$ , Equation A1 gives,  $N^{C}{}_{j} = N^{C}{}_{i} \cdot e^{-\gamma \cdot \Delta t}$ .

766 This relationship and Equation 3 in the text yield an expression for the decomposition

ratio of fine root necromass between times i and j,  $\gamma_{ij}$ , as

768 
$$\gamma_{ij} = 1 - e^{-\gamma \cdot \Delta t}$$
 (A2).

769 Note that  $\gamma$  and  $\gamma_{ij}$  in Equation A2 are different parameters.

Next, let us consider the decomposition process of dead fine roots in an ingrowth core where there is both instantaneous fine root decomposition of  $\gamma \cdot N$  and instantaneous fine root mortality (addition of new dead roots) at a constant level of  $\sigma$ . Here, the process can be described by a differential equation:

774 
$$dN/dt = \sigma - \gamma \cdot N \tag{A3}$$

775 It is well known that the linear first-order differential equation of a form  $dy/dx + P(x) \cdot y =$ 776 Q(x) with two variables x and y has a solution (Kreyszig 1972):

777 
$$y = e^{-\int P(x)dx} \cdot \left\{ \int_{0}^{x} Q(x)e^{\int P(x)dx} dx + C \right\}$$
(A4)

778 where C is any constant. Therefore, Equation (A3) can be solved with a boundary

779 condition,  $N = N_i$  at t = i, as,

780 
$$N = (\sigma / \gamma) + (N_i - \sigma / \gamma)e^{-\gamma \cdot (t-i)}$$
(A5).

relationship,  $\sigma / \gamma = \Delta N / \gamma_{ij} + N_i$ , where  $\Delta N = N_j - N_i$ . Applying this relationship in Equation A5 yields,  $N = \Delta N \cdot (1 - e^{-\gamma \cdot (t-i)}) / \gamma_{ij} + N_i$ (A6). Then, by noting  $\gamma \cdot \Delta t = -\ln(1 - \gamma_{ij})$  from Equation A2, the amount of decomposed dead fine roots between times i and j could be obtained from Equation A6 as,  $d_{ij} = \int_{i}^{j} \gamma \cdot N dt = -\Delta N - (\Delta N / \gamma_{ij} + N_i) \cdot \ln(1 - \gamma_{ij})$ (A7). 

By calculating  $N_i$  with Equation A5 for t = j, then inserting Equation (A2), we obtain the

## 814 **Tables**

823

- 815 Table 1 A version of the decision matrix by Fairley and Alexander (1985) for
- 816 calculating production (g) and mortality (m) of fine roots and decomposition (d) of dead
- 817 fine roots during a measurement interval.  $\Delta B$  and  $\Delta N$  represent change during a
- 818 measurement interval in the pools of live fine root mass and dead fine root mass,
- 819 respectively. Inequalities in the top two rows and those in the first column from the left
- 820 indicate conditions on the values of  $\Delta B$  and  $\Delta N$ . Suggested equations for calculation of
- 821 g, m, and d are given in the Table for specific combination of the values of  $\Delta B$  and  $\Delta N$ .
- 822 The equations in parentheses are assumptions under specific conditions.

	$\Delta B \ge 0$	$\Delta B < 0$	
		$\Delta N >  \Delta B $	$ \Delta B  > \Delta N$
$\Delta N \ge 0$	$g = \Delta B + \Delta N$	$g = \Delta B + \Delta N$	( <i>g</i> = 0)
	$m = \Delta N$	$m = \Delta N$	$m = -\Delta B$
	( <i>d</i> = 0)	( <i>d</i> = 0)	$d = -\Delta B - \Delta N$
$\Delta N < 0$	$g = \Delta B$	( <i>g</i> = 0)	( <i>g</i> = 0)
	( <i>m</i> = 0)	$m = -\Delta B$	$m = -\Delta B$
	$d = -\Delta N$	$d = -\Delta B - \Delta N$	$d = -\Delta B - \Delta N$

848 Table 2 Comparison of fine root production in dry mass during the entire period of 849 experiment estimated by various methods of calculation for the Chamaecyparis obtusa 850 forest at Ryukoku Forest in 2007 and 2010. The 2007 experiment started on July 6, 851 ended on December 21, and lasted for 166 days with four measurement periods. The 852 2010 experiment started on June 22, ended on December 16, and lasted for 177 days with 853 three measurement periods. Fine root ingrowth core was used in 2007 while sequential 854 soil cores were taken in 2010, both with accompanying root decomposition experiment. 855 Production was calculated for three groups of data that differed in characteristics. First 856 group includes only significant differences in  $\Delta B$  and  $\Delta N$ ; second group consists of data 857 of all measurement periods regardless of significance of difference; third includes only 858 data of the first and last soil cores sampled during the experiment.

859

Method used for calc	culation	2007 study	2010 study
		$(g m^{-2} 166d^{-1})$	$(g m^{-2} 177 d^{-1})$
	Using on	ly significant differences	
Decision matrix		$256^{\P}$	O¶
Forward estimate <sup><math>\dagger</math></sup>		$304^{\P}$	$56^{\P}$
Continuous inflow es	stimate <sup>†</sup>	330	$89^{\P}$
Backward estimate <sup><math>\dagger</math></sup>		365	130 <sup>¶</sup>
	Using da	ta from all periods	
Decision matrix		333	493¶
Forward estimate		$243^{\P}$	538
Continuous inflow es	stimate	391	574
Backward estimate		408	622
	Using on	ly first and last sampling	data
$\Delta B + \Delta N^{\ddagger}$		<b>304</b> ¶	<b>493</b> ¶
Decision matrix		304¶	493¶
Forward estimate		<i>304</i> ¶	505
Continuous inflow es	stimate	392	588
		619	744

<sup>\*</sup>Conservative estimate of production in boldface numerals by ignoring decomposition in
Equation 1

886 <sup>¶</sup>Italic numerals indicate values smaller than or equal to the estimate shown with boldface887 numerals for each year.

- 888
- 889

Comparison of annual decomposition rate  $\gamma$  (y<sup>-1</sup>) and annual decomposition 890 Table 3 891 ratio  $\gamma_{ii}$  of dead fine roots reported in various forest ecosystems. Equation A2 was used 892 for the necessary conversion between  $\gamma$  and  $\gamma_{ij}$ . Mean±SE are indicated where 893 appropriate. Data are categorized into four groups depending on the method used for 894 estimation: Litter bags (loose soil contact) is ordinary envelope-type litter bags made of 895 mesh cloth, Litter bags (close soil contact) is the root-impermeable water-permeable 896 sheet-covered and soil-filled ingrowth core—a construction identical to that used in the 897 present study, Trench-plot is a trenched soil block in which root decay is monitored, and 898 Simulation indicates a value derived hypothetically for use in a simulation study. 899 Superscripts indicate references where decomposition rates or ratios were reported: <sup>a</sup>Silver and Miya (2001), <sup>b</sup>Cusack et al. (2009), <sup>c</sup>Osawa A. (unpublished data), 900 <sup>*d*</sup>Publicover (1992), <sup>*e*</sup>Silver and Vogt (1993), <sup>*f*</sup>calculated from monthly  $\gamma_{ij}$  of 0.15 in 901 902 Publicover and Vogt (1993), and <sup>g</sup> sample size not applicable due to simulation. 903 904  $(y^{-1})$ (dimensionless) Number of 905 Site annual  $\gamma_{ii}$ estimated  $\gamma$ γ 906 907 *Litter bags (loose soil contact)* 908 Global mean, broadleaf  $0.44^{a} \pm 0.06$ 0.35 40 909 Global mean, conifer  $0.17^{a} \pm 0.02$ 0.15 10  $LUO^{\dagger}$ , Puerto Rico  $1.06^{b} \pm 0.28$ 910 0.65 4 GSF<sup>†</sup>. Puerto Rico 911  $0.42^{b} \pm 0.07$ 0.34 4 912 913 *Litter bags (close soil contact)* this study at  $RKF^{\dagger}$ , 2007 2.61<sup>¶</sup>±0.53 914  $0.87^{\text{l}}\pm 0.07$ 5 this study at  $RKF^{\dagger}$ , 2010 1.30<sup>¶</sup>±0.28 915  $0.65^{\text{l}}\pm 0.05$ 4 WBNP<sup>†</sup>, young jack pine 916  $0.24^{c} \pm 0.03$  $0.21 \pm 0.02$ 2 WBNP<sup>†</sup>, mid-age jack pine 917  $0.30^{\circ} \pm 0.05$  $0.26 \pm 0.03$ 5 WBNP<sup>†</sup>, old jack pine 918  $0.16^{\circ} \pm 0.04$  $0.14 \pm 0.04$ 5 919 920 Trench-plot NJPB<sup>†</sup>, pine  $0.58^{d}$ 921 1 0.44 NJPB<sup>†</sup>, oak 922  $0.56^{d}$ 0.42 1

923	NJPB <sup>†</sup> , Ericaceous	$0.78^d$	0.54	1
924	LUQ <sup>†</sup> , lower plot	$0.8^{e}$	0.55	1
925	LUQ <sup>†</sup> , upper plot	$0.9^{e}$	0.59	1
926	$LUQ^{\dagger}$ , control forest	$0.4^{e}$	0.32	1
927				
928		Simulation		
929		$1.95^{f}$	$0.85^{f}$	_g
930				

931 <sup>†</sup>Abbreviations of names of places (locations): LUQ: Luquillo Experimental Forest,

932 Puerto Rico (18°N, 5°W); GSF: Guanica State Forest, Puerto Rico (17°N, 65°W); RKF:

933 Ryukoku Forest, Japan (34°N, 135°E); WBNP: Wood Buffalo National Park, Canada

934 (68°N, 113°W); NJPB: New Jersey Pine Barrens, USA (39°N, 74°W).

935 Calculation was based on field data from experiments each consisting of several periods

that lasted 30—92 days during non-winter seasons.

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938

## 939 **Figure captions**

940 Fig. 1 A compartment model representing the mass balance of organic matter that is 941 derived from live fine roots in the soil volume. The boxes express the stock: *B* is live 942 fine roots and N is dead fine roots and organic matter of fine root origin. The arrows 943 indicate mass flow into, between, or out of the compartments:  $g_{ij}$  is fine root ingrowth;  $m_{ij}$ 944 is fine root mortality; and  $d_{ij}$  is decomposition of dead organic matter between the times i 945 and j. The amounts of live fine roots at times i and j are expressed as  $B_i$  and  $B_j$ , 946 respectively, in the text.  $N_i$  and  $N_j$  express the amounts of necromass at times i and j, 947 respectively.  $\Delta B$  and  $\Delta N$  represent changes in fine root biomass and necromass 948 between times i and j.

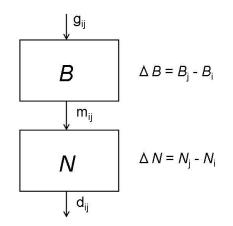
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Fig. 2 A sequence of soil core installations into the soil horizon (open circles) and recovery or soil core sampling to measure fine root biomass and necromass (filled circles) of a set of three soil cylinders used to evaluate fine root ingrowth, mortality, and decomposition simultaneously between times i and j, where 0 < i < j. For ingrowth cores, the cores A and B are installed at the same time (time zero) but are recovered at different times. For sequential soil sampling, soil cores A and B are collected at times i and j, respectively. Litter bag C is the 'root-impermeable water-permeable (RIWP) sheet'-covered core installed with known mass of dead fine roots added at time i, andrecovered at time j with core B.

959

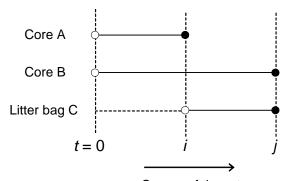
960 Fig. 3 Estimated values of fine root decomposition (d<sub>ii</sub>), mortality (m<sub>ii</sub>), and 961 production (g<sub>ii</sub>) for the ingrowth core experiment of 2007. The estimates are compared 962 to those calculated by the decision matrix (DM, cross pattern), forward estimate (F, left-963 slanting pattern), continuous inflow estimate (C, black), and backward estimate (B, right-964 slanting pattern). Vertical bars indicate the upper 95% confidence limits based on the 965 bootstrap method. For the decision matrix method, all decomposition estimates are zero 966 except for the fourth period that ended on December 21. Note that the vertical scale is 967 different for fine root production, g<sub>ii</sub>. 968 <sup>a</sup>The values of fine root production were set at zero due to the negative values obtained. 969 See text for further explanation. 970 971 Fig. 4 Estimated values of fine root decomposition (d<sub>ii</sub>), mortality (m<sub>ii</sub>), and 972 production  $(g_{ij})$  for the sequential soil core sampling of 2010. The estimates are 973 compared to those calculated by the decision matrix (DM, cross pattern), forward 974 estimate (F, left-slanting pattern), continuous inflow estimate (C, black), and backward 975 estimate (B, right-slanting pattern). Vertical bars indicate the upper 95% confidence 976 limits based on the bootstrap method. For the decision matrix method, all 977 decomposition estimates are zero. Note that the vertical scale is different for fine root 978 decomposition, d<sub>ii</sub>. 979 980 981 982 983 984 985

# 986 Figures



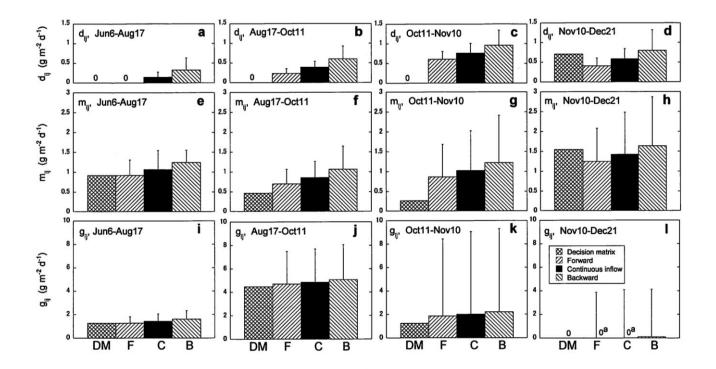
- **Fig. 1**

- //1



Course of time, t







- **Fig. 3**

