Original research article

A new method for placing and lifting root meshes for estimating fine root production in forest ecosystems

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Abstract: We describe a new and easy technique for placing and lifting root meshes to estimate fine root production in forest ecosystems. The method improves upon previously proposed mesh placement techniques by using a sharp stainless steel blade and two thin stainless steel sheets to insert mesh more easily and accurately in the soil, and utilizing a narrow garden spade to lift the soil block containing the mesh. The proposed technique takes significantly less time than the widely used ingrowth core method, causes minimal disturbance to the soil, and requires only simple equipment. The detailed documentation of the method provided herein should improve estimations of fine root production in forest ecosystems.

Keywords: fine root production, ingrowth core method, root mesh, tree roots

Introduction

Fine roots are essential not only for water and nutrient uptake but also for their contribution to carbon cycling in forest ecosystems (Brunner and Godbold 2007). However, our understanding of the growth dynamics of fine roots is still limited because of technical and methodological difficulties (Majdi et al. 2005, Finér et al. 2007, Noguchi et al. 2007). Estimates of both fine root biomass and production are needed to calculate root turnover, which is one of the least understood aspects of the global carbon cycle (e.g., Gill and Jackson 2000).

Several direct and indirect methods for estimating fine root production have been developed; these include sequential soil coring, ingrowth core, minirhizotron imaging, and element budget analysis (i.e., C and N budgets; Majdi et al. 2005, Hendricks et al. 2006). However, measurements of root production generally have a high degree of error (e.g., soil coring), produce changes in the chemical and physical properties of the soil (ingrowth core), or are time and labor intensive (e.g., minirhizotron imaging, Godbold et al. 2007). The root mesh method has been proposed as an alternative technique that overcomes these problems (Fahey and Hughes 1994, Jentschke et al. 2001, Godbold et al. 2003, 2007, Montagnoli et al. 2007). Using this technique, root production is estimated by placing a mesh vertically into forest soil for a specific period of time and then measuring the number and weight of roots that grow through the mesh. The procedure is much easier than other methods, requires only simple equipment, and causes minimal soil disturbance (Godbold et al. 2007). However, very few studies have considered this sampling technique, and the methods of mesh placement and lifting are neither well developed nor well documented. Therefore, a detailed description of the methodological protocol is needed for wider application of this sampling strategy. Here, we propose and document a novel approach for both placing and lifting root meshes to estimate root production, and compare the new strategy to previous root mesh practices and to the ingrowth core method.

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Materials and Methods

Mesh placement

We placed nylon meshes vertically into forest soil in a *Cryptomeria japonica* stand to a depth of 20 cm using both the conventional method (Jentschke et al. 2001, Godbold et al. 2003, 2007, Montagnoli et al. 2007) and the newly proposed technique.

The procedure following the conventional method was as follows (Fig. 1):

1. A straight and sharp stainless steel blade (10 cm × 45 cm, 2 mm thick) was pushed into the ground to a depth of 20 cm (Figs. 1A, B1, B2, C1, C2).
2. The blade was removed, leaving a vertical slit in the soil (Figs. 1B3, C3).
3. The blade was wrapped in a sheet of nylon mesh (10 cm × 40 cm, 1 mm mesh size; Figs. 1B4, C4).
4. The blade wrapped with the mesh was inserted into the slit to a depth of 20 cm (Figs. 1B5, B6, C5, C6).
5. Only the blade was taken out, leaving the mesh inserted vertically in the soil.

The procedure following new method was as follows (Fig. 2):

1. A straight stainless steel blade (10 cm × 30 cm, 2 mm thick) was pushed into the ground to a depth of 20 cm (Figs. 2A, B1, B2, C1, C2).
2. A nylon mesh (10 cm × 20 cm, 1 mm mesh size) was placed between two thin straight stainless steel sheets (10 cm × 30 cm, 1 mm thick) attached to a single handle (Figs. 2B3, C3).
3. The apparatus containing the mesh was inserted gradually into the slit, along the inserted blade (Figs. 2B4, C4).
4. The blade was removed slowly from the slit (Figs. 2B5, C5).
5. The handle of the apparatus made of two thin stainless steel sheets was removed and each sheet was individually extracted from the slit (Fig. 2B6), leaving the mesh inserted vertically in the soil (Fig. 2C6).

To compare the time required to place the mesh using each method, we performed a study in a 51-year-old *C. japonica* stand in Nishinomiya (34°49'N, 135°16'E), Hyogo, Japan. Two researchers with previous experience in mesh placement tested each method three times on the same day. The order of the method trials was randomized, and the weather on the day was clear. The mean diameter at breast height (DBH) was 31 cm and tree density was 1,100 trees per ha. The soil was classified as brown forest soil (Forest Soil Division, 1976), which corresponds to an Inceptisol in the U. S. Department of Agriculture Soil Taxonomy (Soil Survey Staff, 1998). The parent rock was rhyolite. We selected six *C. japonica* trees in a plot of 20 m × 20 m. One nylon mesh at a time was inserted vertically using each method at distances of 1 m from each tree (n = 6) and the time it took to complete each placement was recorded in seconds.

Lifting root meshes

To estimate fine root production, the number and weight of roots that have grown through the mesh should be measured after a set time, e.g., 1 or 2 years after placement. The mesh is two-dimensional, but a three-dimensional soil block including the mesh should be extracted to obtain an estimate of fine root production. We developed an easy method to excavate the soil block using a narrow garden spade (Horito-ruzou, AD620, Aida, Niigata, Japan, Fig. 3A) that fits the sampling position. The size and shape of the spade is similar to that of the mesh. The step bars (red arrows in Fig. 3A) on the spade make it easier to push the spade into the ground and slice through the roots. The procedure for sampling was as follows:

1. Two curvilinear lines against both mesh edges were cut in the soil to a depth of 20 cm using the spade (lines 1-1 and 1-2 in Fig. 3B).
2. Two curvilinear lines parallel to the mesh at a distance of about 10 cm were cut to a depth of 20 cm (lines 2-1 and 2-2 in Fig. 3B).
3. Two additional lines about 5 cm from the mesh were cut (lines 3-1 and 3-2 in Fig. 3B, Figs 3C1, 3D1). The aim of steps 1-3 was to cut the roots that had grown through the mesh and prevent them from falling out of the mesh.
4. The soil between lines 2-1 and 3-1 was removed to a depth of 20 cm (area no. 4 in Fig. 3B, Figs. 3C2, C3, D2, D3). Roots that grow below the mesh (around a depth of 20-25 cm) can be cut with pruning shears.
5. The soil on line 3-2 was cut again using the spade (Figs. 3C4, D4), and the spade with the soil block was pulled out in the direction of line 3-1.
6. The soil block was removed from the spade (Figs. 3C5, C6, D5, D6).

It should be noted that this procedure was conducted in a 53-year-old *C. japonica* stand in Wachi (35°19'N, 135°27'E), Kyoto, Japan, soon after mesh placement, and thus we did not have roots that had grown through our mesh samples. The mean DBH was 30 cm and tree density was 1,200 trees per ha. The soil was classified as brown forest soil (Forest Soil Division, 1976), and the parent rock was mudstone.

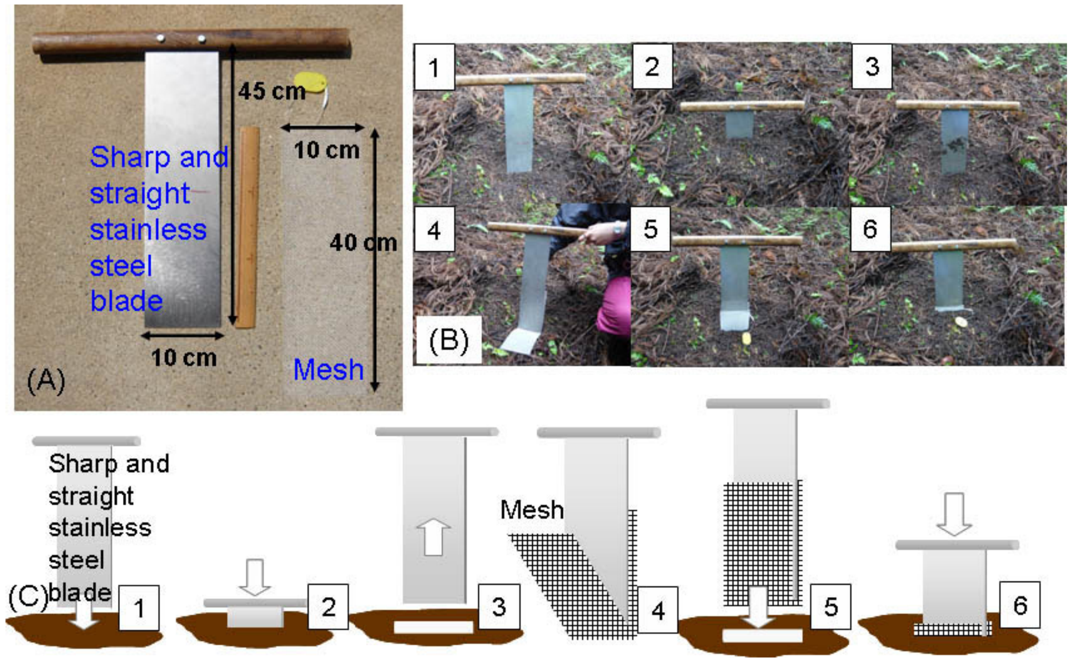

Fig. 1. The conventional method of placing a mesh proposed in earlier reports (Jentschke et al. 2001, Godbold et al. 2003, 2007, Montagnoli et al. 2007). (A) A straight and sharp stainless steel blade for making the soil slit and a nylon-mesh sheet, (B) photos of each step in the procedure, and (C) schematic illustration of the procedure.

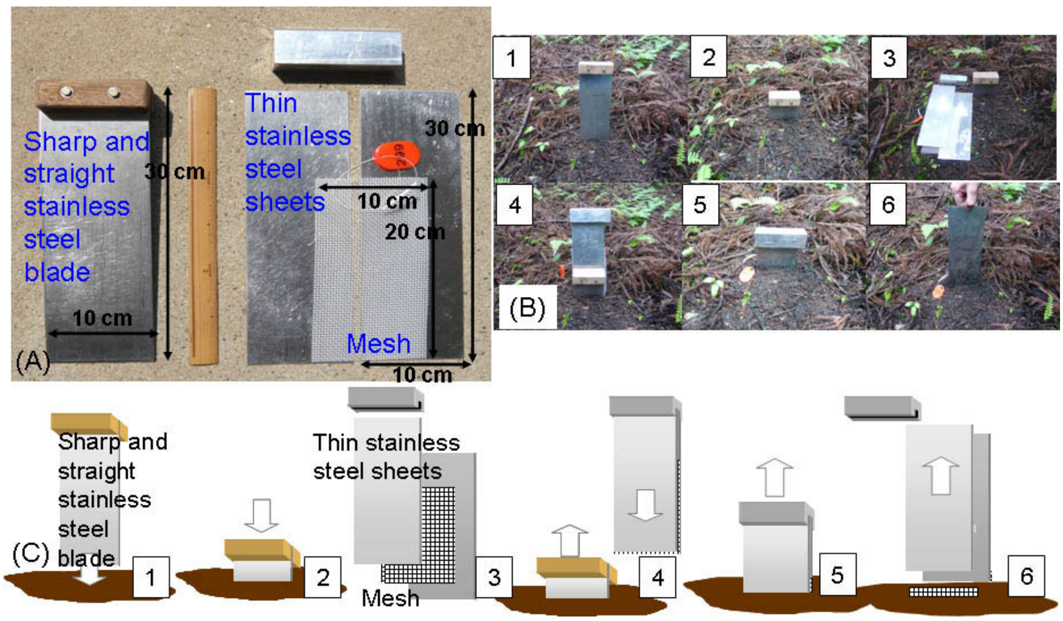

Fig. 2. The new method for mesh placement. (A) A straight and sharp stainless steel blade for making the soil slit, two thinner stainless steel sheets for placing the mesh, and a nylon mesh sheet, (B) photos of each step in the procedure, and (C) schematic illustration of the procedure.

Comparison of the new mesh placement method and the ingrowth core technique

To compare the time required for each technique, we performed a study in the *C. japonica* stand in Wachi. A plot of 10 m × 10 m was established and ingrowth cores (nylon mesh cylinders 5 cm in diameter × 20 cm deep, 1 mm mesh size) and meshes were inserted on a

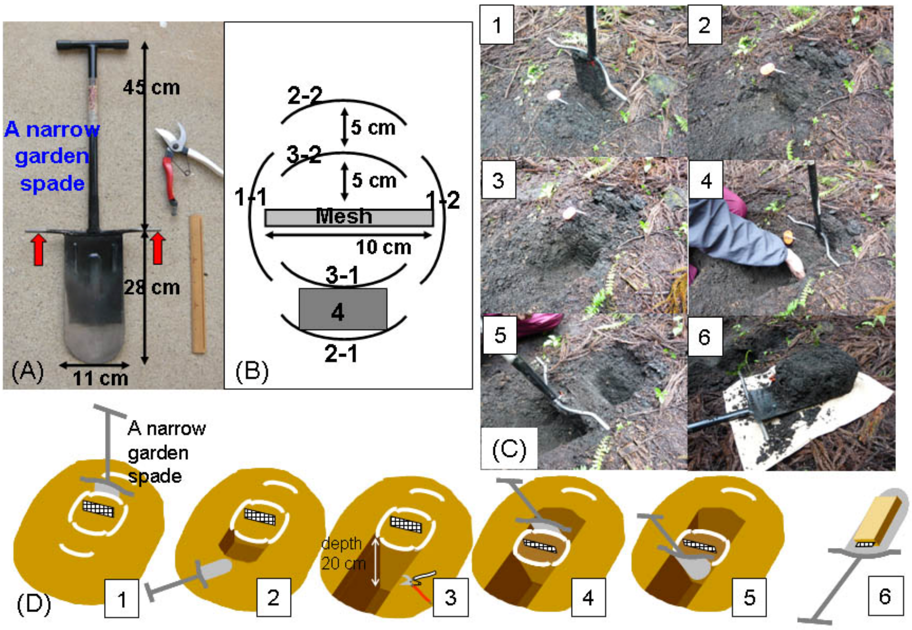

Fig. 3. Lifting of the soil block including the root mesh using a narrow garden spade. (A) The spade for sampling soil blocks, (B) cutting position using the spade, (C) photos of each step in the procedure, and (D) schematic illustration of the procedure.

grid in 1-m intervals. Ingrowth cores were installed in holes where the soil had been removed with a corer, and the cores were filled with root-free soil from the same stand. Two researchers who had prior experience with the mesh placement tested each technique five or six times on the same day. The order of testing the methods was randomized and the weather on the day was clear. A total of 11 root meshes and 11 ingrowth cores were installed (n = 11), and placement times were recorded in seconds.

Results and Discussion

When the conventional method used to insert the mesh, some soil particles typically dropped into the slit as the blade was taken out. In such cases, it took longer to place the mesh because it was difficult to insert. At other times, the mesh did not fold correctly as it was inserted into the soil, suggesting that the resulting mesh area (10 cm × 20 cm) sampled was not precise. Mesh size (ca. 1 mm mesh) can also vary depending on how well the mesh folds in the soil. In contrast, when our new method was used to insert the mesh, soil did not easily fall into the slit because the mesh was installed using two thin stainless steel sheets pushed along the blade before removing the blade. The mesh size was always uniform because the mesh did not need to be folded. The mean placement time of the root mesh in the forest soil of the *C. japonica* stand was 152±85 and 102±59 s (mean ± SE, n = 6) using the conventional and new method, respectively. The differences were not statistically significant (*t*-test, t= 1.18, p=0.26). These results indicate that the new method is superior to the conventional method in terms of the accuracy of the soil depth and the mesh size at the time of mesh placement.

We showed that soil blocks that include the mesh can be lifted using a narrow garden spade (Fig. 3). The block size was approximately 12 cm × 8 cm, with a depth of 25 cm. The mesh containing the newly grown roots can be lifted from the soil block from a definite distance from each side of the mesh; if a distance of 2 cm were used, then the final block for estimating root production would be 10 cm × 4 cm, with a depth of 20 cm. The remaining roots (those that grew through the mesh) are then extracted from the soil block to determine their dry weight. If the mesh of 10 cm width with 2 cm on each side were harvested 1 year after the placement, we could calculate the fine root production (g m^{-2} $year^{-1}$) as follows;

Fine root production (g m^{-2} $year^{-1}$)

$$= \frac{RDW}{Wm \times (2 \times Des)/10000}$$

where *RDW* is the dry weight of newly grown roots that have gone through the mesh (g), *Wm* is the wide of the mesh (10 cm) and *Des* is the distance from each side of the mesh (2 cm).

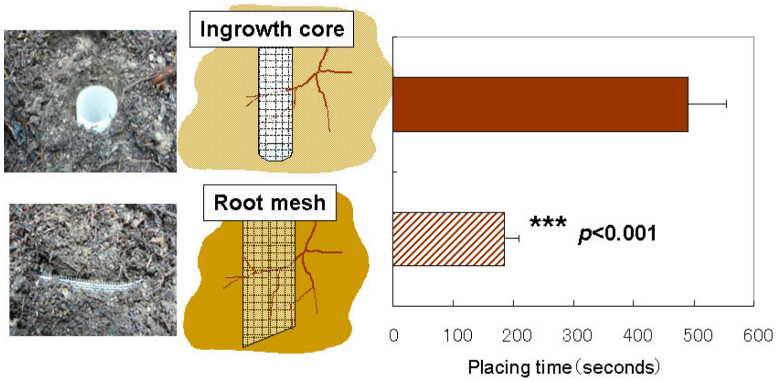

Fig. 4. Average time (mean+1S.E., n=11) for placing an ingrowth core and a root mesh in the *Cryptomeria japonica* stand. ***p<0.001; significant difference between the methods calculated using a *t*-test.

We also compared the placement time of the new method to that of the ingrowth core technique, which is widely used to estimate fine root production in forest ecosystems (Majdi et al. 2005, Hendricks et al. 2006). The mean placement time using the ingrowth core method in the *C. japonica* stand was 491±64 s, while that using the new method was 185±24 s (mean ± S.E., Fig. 4), which represents a significant difference (*t*-test, t = 4.47, p<0.001). The ingrowth core method requires preparing not only the cylinder-shaped core but also the soil before placement, and therefore takes much more time. It also alters nutrient availability and the soil structure when soil is placed in the cores (Majdi et al. 2005). The root mesh method causes minimal disturbance to the soil because the mesh is set simply and vertically in a soil slit that is a few millimeters in width (Godbold et al. 2007), and therefore improves the estimation of fine root production.

However, there are some limitations to using the new method. Roots are cut when the slit is made to place the mesh, and this can stimulate root growth. In addition, in contrast to minirhizotron imaging, it is impossible to obtain temporal information on root growth and mortality. These are also true with the ingrowth core method (Majdi et al. 2005). Even though fine root production estimates obtained using the ingrowth core and root mesh methods are comparable (Jentschke et al. 2001, Godbold et al. 2003), the lower estimation of fine root production using the ingrowth core method is a potential error (e.g., Hendricks et al. 2006). Finally, roots in soil blocks that did not grow through the mesh cannot be used to estimate fine root production.

Our new method of placing and lifting mesh is superior to the ingrowth core method because of its simplicity and minimal disturbance to the soil at the time of the mesh placement. The detailed methodological protocol provided herein will make it easier to estimate fine root production. We have started a series of experiments to determine if the newly developed root mesh method gives comparable results to the ingrowth core method in *C. japonica* forests in Japan, where few data on fine root production are available (Noguchi et al. 2007). If these experiments are successful, a significant number of new estimates of fine root production will accumulate in the near future.

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The author's photo with Onnetou-mountain at Ashoro town, Hokkaido, where the 30th JSRR meeting was held in May 2009. **From left to right**: **Prof. Leena Finér** is interested in forest ecology especially about the environmental impacts of forestry practices on ecosystem process and functions. She studies mainly carbon and nutrient pools and fluxes in belowground parts of forest ecosystems. **Saori Fujii** studies the relationship between fine roots and springtails in forest ecosystem. **Dr. Kyotaro Noguchi** is a senior researcher of Forestry and Forest Products Research Institute, Japan. His research interests are focused on nutrient dynamics and fine root turnover in forested ecosystems. **Naoki Makita**'s research interests are carbon and nitrogen cycling in forest ecosystems, especially fine root respiration and root dynamics. He loves roots. Study subject of **Dr. Takuo Hishi** is relationship between characteristics of tree fine root system and soil animals in relation to forest ecosystem productivity. **Dr. Mizue Ohashi** studies carbon cycling in forest ecosystems. She wants to know how carbon dynamics is affected by biological activities, such as soil microbial condition, root growth, animal behavior and anthropogenic disturbances. **Dr. Yasuhiro Hirano** has focused on physiological responses of fine roots of forest trees as an indicator of soil acidification and has been trying to establish a net work of tree root research in Japan.