Short report

The development of an optical scanner method for observation of plant root dynamics

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Abstract: In order to achieve continuous monitoring of the rhizosphere, we developed a simple method that uses a wide-view optical scanner inserted into the ground. The scanner system facilitates the analysis of image data and allows continuous monitoring by automating the capture and by fixing the position of the optical scanner. The system was tested in the laboratory and installed under CO_2 measurement system in the field. Clear images were obtained, suggesting the possibility of analyzing live-dead-decomposition root dynamics continuously.

Keywords: scanner, root turnover, root production, root decomposition, image analysis.

Introduction

Root dynamics such as root production, death, and decomposition are directly related to the carbon dynamics of forests. There have been many trials to separate root respiration from soil respiration for the understanding of forest carbon cycle (Hanson et al. 2000), but there are not perfect methods because each method of measuring root has done with specific difficulties of some disturbances or uncertainties. Thus, complex approach is needed for the credible results. In particular, fine roots, because of their immediate response, can be a good indicator of environmental changes (Hirano et al. 2007). Noguchi et al. (2007) reviewed studies on biomass and the productivity of fine roots in Japanese forests and concluded that future studies were needed on fine root dynamics in relation to individual root architecture, anatomy, and physiology. Moreover, the processes related to fine roots are thought to play a very important role in the circulation of carbon and nutrients in forest ecosystems (Gill and Jackson 2000) because of the high productivity of fine roots (Satomura et al. 2007, Vogt et al. 1982) and their high respiration rate (Pregitzer et al. 1998). For example, it has been reported that in a deciduous forest in Kyoto, Japan, the fine roots that constituted only about 16% of the total root biomass provided more than half of the respiration (Dannoura et al. 2006a). In previous study, we developed the system for measuring separated root respiration continuously (Dannoura et al. 2006b). And in this study, we developed a method to observe fine root dynamics simultaneously in parallel with measuring root respiration using wide-view optical scanner.

There are disruptive and non-disruptive ways to research roots in their natural states. Disruptive methods such as digging, using monoliths, augers and cores, are mainly used in studies of root structure and production ecology. Non-disruptive methods such as using rhizotrons, minirhizotrons, and radioactive tracers are mainly used for continuous observation of root extension or to investigate the distribution of live roots. The minirhizotron method, which uses a transparent tube (around 65 mm in diameter) inserted into the soil, is a well-established technique in long-term root dynamics studies (Johnson and Mayer 1998, Satomura et al. 2001). This method has the advantages of minimizing disturbance and being usable in long-term root dynamics measurement. To obtain a string of images, the observer must put a CCD camera into the transparent tube manually. The size of a single image is 16×18 mm and the observer can capture connected images by moving the camera along the tube (Minirhizotron BTC-100X; Bartz Technology Corporation, USA). However, mini-rhizotron systems are very expensive and it is difficult to observe whole root systems because the measurable

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area of single tube is small. Also, this method requires the researcher to bring the camera to the research site where monitoring is being done. The intervals between images vary according to the observer. There is the possibility of missing root turnover because of the long gap between observations and the amount of production and dead root between captures is not observed, so it can be underestimated (Rygiewicz et al. 1997). Therefore, the observer must take care to minimize the intervals between images. In fact, a system for automating image capture for mini-rhizotrons has recently been developed: CI-600 Root scanner (CID, Inc, WA, USA). Bigger images can be obtained with root windows, which are transparent viewing planes installed in soils. Compared to minirhizotrons, the viewing area of root windows is usually much larger and not curved. They are suitable for monitoring root growth and performing detailed studies of individual roots. However they cause a bigger disruption to the soil and installation can be complicated. A camera or scanner can be used to obtain images, however there are no products currently available for automatically capturing images by the root window method.

It can be challenging to make progress in evaluating fine-root dynamics in light of these difficulties. Explaining the production activities of fine roots requires an evaluation of various processes, such as the amount of production of fine roots, the seasonality of production, and the process of decomposition. For such an evaluation to take place, it is necessary to capture successive images covering a relatively wide area and to analyze the differences among the images. With this in mind, we developed an optical scanner system to respond to the recognized need to obtain more refined root images on a more frequent basis. This observation was done in the laboratory and in the field. After we certified the performance of the system, we establish the system in the field and set up the CO_2 efflux measurement system that can measure only from root. CO_2 efflux and environmental factor were measured simultaneously.

Materials and Methods

We used a thin-type flat-head scanner with a clear cover that can be buried in the soil and connected to a personal computer (PC). The scanner can be placed horizontally, vertically or obliquely depending on the purpose or the image sought (Fig. 1). There are two types of scanners: Contact Image Sensor (CIS) and Charge Coupled Device (CCD). The CIS-type scanner is very thin, its power can be supplied through a USB cable from a PC, and warm-up time is eliminated. The CCD-type scanner is thicker than the CIS and it reads the image through the lens after reflected light is consolidated in a mirror. It differs from CIS in its clear interpretation of rugged objects. For experiments that took place in the laboratory, the CIS scanner could be covered in a thin material such as a plastic bag. However, a thicker acrylic cover had to be used to protect the equipment during the outdoor experiments, which tended to cause objects to be out-of-focus, so the CCD scanner is recommended for fieldwork.

First, we used the scanners to observe a root system under laboratory conditions to validate temporal observations of potted plant roots. *Ophiopogon japonicus* (L.f.) Ker Gawl. and *Glycine max* (L.) Merr. were used as the sample plants. *Ophiopogon japonicus* (L.f.) Ker Gawl. is commonly found as under vegetation in the Japanese countryside. We used mixed humus soil and nursery soil material (Kanumatsuchi, pumice from the Kanuma region of Japan) at a ratio of three to one. The pot was put outside and watered once a day. We used a CIS scanner (LiDE60 and 500F, Canon, Japan) with an image size of 216×297 mm and a height of 25 mm with the cover removed. The scanner was placed in a plastic bag for waterproofing connected with PC by USB cable and then buried on an angle. We took one image per day in the *Ophiopogon japonicus* (L.f.) Ker Gawl. pot and one every 4 hours in the *Glycine max* (L.) planter.

Next, we undertook field observations at Yamashiro Experimental Forest, located in a hilly and mountainous region in Kyoto Prefecture, Japan (34º47'N, 135º51'E). The soil is classified as a Dystric Cambisol by WRB-classification and is derived from weathered granitic parent materials. We made an acrylic box for the scanner so that it would be protected from rain in the field. We used a CCD scanner with an image size of 216×297 mm (GT-F650, Epson, Japan) because it has a greater focal length than a CIS scanner. The scanner was inserted in the acrylic box without the cover. One side of the acrylic box was closed with a screw. A USB cable and power supply cable were run from a small port on the cover and a PC was connected in storage. To separate the roots from organic soil, all soil in the A horizon was carefully removed using a portable electric vacuum cleaner, leaving only the living roots. Care was taken to avoid damaging any roots during this process. The acrylic box containing the scanner was inserted between the A and B horizons at about 70 mm in depth. We carefully replaced the soil with an equal depth of weathered granitic soil (obtained from a gardening supply store) that was similar to the original A horizon at the study site. Images could be obtained automatically by software. We used UWSC (Ver. 4.1) which is free software for recording and playback of input by mouse or keyboard. Observers can configure the frequency of capture and change the settings at their convenience. In this study, we set the interval to every

30 minutes. We set up the scanner in the forest soil on February 12, 2007 and started monitoring on July 3, 2007.

We added the flux chamber on the top of soil on the scanner system in the field (Fig.1). We measured root respiration on the assumption that the CO_2 efflux is from root only, because organic soil was not included in the chamber. Detail of the system for respiration measurement was shown in our previous study (Dannoura et al. 2006b). We measured root respiration every one hour and soil temperature and moisture (ECH2O probes, Decagon, USA) in 50 mm depth at the same time.

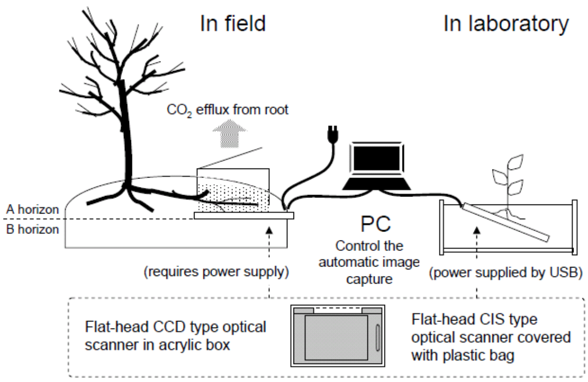

Fig. 1. Outline of the scanner system. Both CCD and CIS type scanner are available according to research objects. In the field measurement, we tried to measure root dynamics and root respiration at the same time. Root system was concentrated in a thin A horizon and soil was removed from the A horizon with minimal disturbance of the remaining roots. A scanner was inserted between the A and B horizons. Weathered soil (obtained from a gardening supply store) that produced a near-zero CO_2 efflux replaced the removed forest soil. Automated open-close chamber was put on the plot.

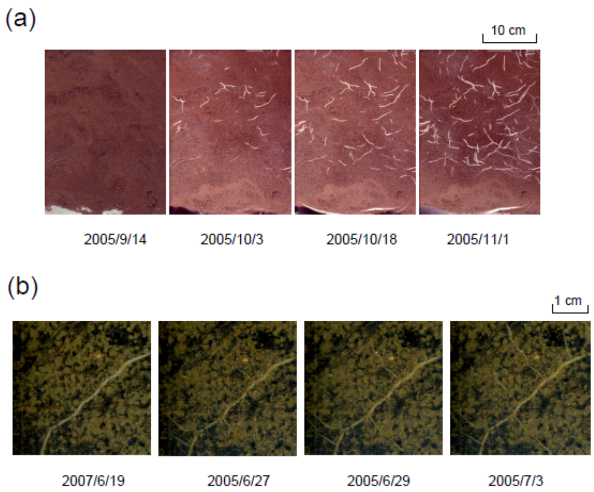

Fig. 2. *Ophiopogon japonicus* (a) in laboratory experiments and *Quercus serrata* in field experiments (b).

Results and Discussion

Figure 2 shows (a) an example of a series of whole images of the roots of *Ophiopogon japonicus* (L.f.) Ker Gawl. from the laboratory experiments and (b) a detail taken from images of *Quercus serrata* Thunb. from the field experiments. From the temporal measurements, the process of root production can be clearly seen. Our observations can catch the elongation rate over a short period; for example, the rate of growing of root tips of *Quercus serrata* Thunb. was analyzed from July 3, 2007 at 16:31 to July 5, 2007 at 02:31 using positioning system in PhotoShop, every 30 minutes (Fig. 3). In this period, the average extend rate was 0.82 mm h^{-1} and the maximum rate was 3.41 mm h^{-1}. The root extended more than 25 mm within 34 hours. Using a system to measure root respiration independently, seen in Fig.3, change in soil temperature was small (within 1 degree) during measurement period, root elongation seems to increase with amplitude range.

Dannoura et al. (2006b) reported that root respiration shows seasonal variations and showed responses to soil temperature and soil moisture from temporal measurements. They also pointed out that, root respiration during the growing period was higher than that during other periods, perhaps due to phenological influences such as fine root dynamics. The distribution and phenology of fine roots are a crucial factor in the carbon dynamics of forests and above all, influence of fine root growth and root litter occurrence upon CO_2 efflux is one of key point charts in evaluation of below ground carbon dynamics. Combined measurement of CO_2 efflux from root or root litter and image analysis of root dynamics might come up with useful information about mentioned propositions. High frequency observations seem to be useful for catching the response to phenological events and the process of root dynamics.

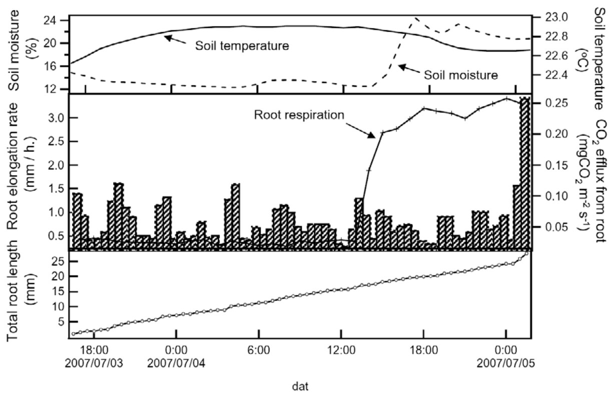

Fig. 3. Temporal change of root tip in *Quercus serrata*. One scale unit on the X axis means 30 min. The upper is extend rate of root every 30 min., the under is total length of root.

Table 1. Comparison of the characteristics between three kind of method for observation of root

Characteristics	Methods		
	Scanner	Minirhizotron	Root Window
Expenditure (10000 yen)	<20	300-800	<20
Usage in laboratory	+++	+++	+
Usage in forest	++	+++	+++
Repetition of the data	+++	+++	+
Long-term research	++	+++	+++
Less disturbance of the survey site	+	+++	+
Size of the observation screen	+++	+	+++
Power supply	necessary	necessary	not necessary
Easy automation	+++	++	+

Symbols are as follows: +++; optimal, ++; suitable, +; feasible

Though care is needed during scanner installation for continuous measurement, a large memory is needed for high frequently image capturing, and stability of power supply are needed for long-term measurement, our scanner method will help to resolve many unexplained factors related to root dynamics, such as production and elongation of roots, death and decomposition process of roots, the seasonality and diurnal cycles of the turnover rate, and the response to precipitation and temperature.

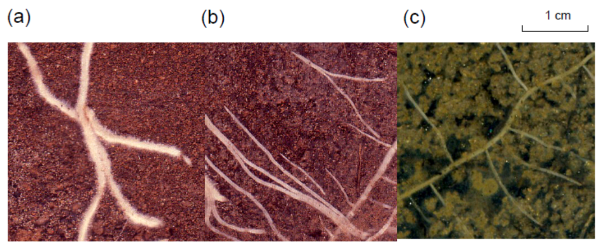

Fig. 4. Magnification of the scanner image. The root of *Ophiopogon japonicus* (a) has root hair, but that of *Glycine max* (b) does not. *Ophiopogon japonicus*'s. *Quercus serrata* (c) has a very thin root, a little like a fishbone.

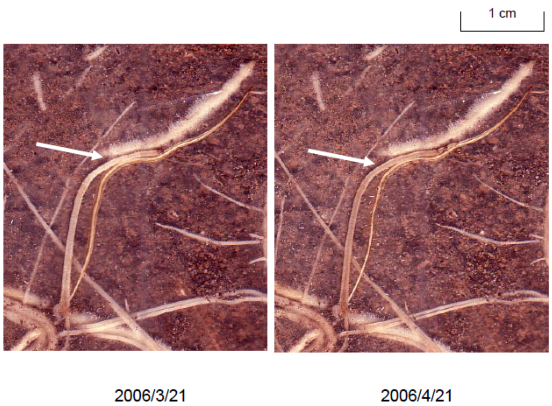

Fig. 5. Color change in a root of *Glycine max*. There are two species of roots in the images. The root of *Glycine max* (indicated with the arrow) extending to the center was white (21/Mar./2006) and after time it changed to brown (21/Apr./2006).

Figure 4 shows examples of images of the root of (a) *Ophiopogon japonicus* (L.f.) Ker Gawl. and (b) *Glycine max* (L.) Merr. in laboratory experiments, and (c) *Quercus serrata* Thunb. in the field experiment. The resolution of the scanner was high (e.g. 1200×2400 dpi) in this case. The resolution depends on the scanner and the software, and it can be adjusted according to the purpose and objectives of the observations. We can see root hairs in Fig 4(a). The root of *Quercus serrata* Thunb. could be observed even though it is very fine. We could enlarge the image because of the high magnification. A change in root color indicates a change in root condition, such as lignification in a white root and root death. Fig. 5 shows a change of root color to brown. It can be particularly difficult to investigate the amount of root litter. Litter from aboveground can be caught by litter traps, but this method cannot be used underground. However root litter is an important parameter in soil carbon dynamics. Methods for measuring root litter are therefore much needed.

The visible range was enlarged to A4 size by the scanner. A minirhizotron of a small size is good for ensuring minimum disturbance, but its visible area is quite small, hence the probability of capturing root dynamics is low. A scanner has a wide area of visibility and a low risk of missing growing roots. It has the advantage of being able to capture the total image and the connection to the soil, mycorrhiza, etc. The width of the visible area is useful when estimating root death, root biomass, and root production, too. However, this system has the same disadvantages as root windows: as the area covered is bigger than with a minirhizotoron, there is a bigger disturbance. The boundary layer is built on the scanner and it is different from natural conditions. Methods need to be selected in line with the objectives of the research. Table 1 shows the advantages and disadvantages of the root scanner method, the minirhizotron method, and the root window method.

The images from the scanner can be used in "WinRHIZO" (Regent Instruments Inc.), similar to those from a minirhizotoron. This software can effectively analyze various factors such as root length and root area, count tips, and perform branching and color analysis. It is necessary to use various programs to automatically analyze the huge volumes of data from these types of observations. However, the configuration of a suitable threshold level has been left for future work.

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References

Dannoura M, Kominami Y, Tamai K, Goto Y, Jomura M, Kanazawa Y 2006a Short-term evaluation of the contribution of root respiration to soil respiration in a broad-leaved secondary forest in the southern part of Kyoto Prefecture. J. Agric. Meteorol. 62: 15-20. (in Japanese with English abstract)

Dannoura M, Kominami Y, Tamai K, Jomura M, Miyama T, Goto Y, Kanazawa Y 2006b Development of an automatic chamber system for long-term measurements of CO_2 flux from roots. Tellus B 58: 502-512.

Gill RA, Jackson RB 2000 Global patterns of root turnover for terrestrial ecosystems. New Phytol. 147: 13-31.

Hanson PJ, Edwards NT, Garten CT, Andrews JA 2000 Separating root and soil microbial contribution to soil respiration: a review of methods and observations. Biogeochemistry 48: 115-146.

Hirano Y, Mizoguchi T, Brunner I. 2007 Root parameters of forest trees as sensitive indicators of acidifying pollutants: a review of research of Japanese forest trees. J. For. Res.12: 134-142.

Jonson MG, Meyer PF 1998 Mechanical advancing handle that simplifies minirhizotron camera registration and image collection. J. Environ. Qual. 27: 710-714.

Noguchi K, Konopka B, Satomura T, Kaneko S, Takahashi M 2007 Biomass and production of fine roots in Japanese forests. J. For. Res. 12: 83-95.

Pregitzer KS, Laskowski MJ, Burton AJ, Lessard VC, Zak DR 1998 Variation in sugar maple root respiration with root diameter and soil depth. Tree Physiol. 18: 665-670.

Rygiewicz PT, Johnson MG, Ganio LM, Tingey DT, Storm MJ 1997 Lifetime and temporal occurrence of ectomycorrhizae on ponderosa pine (*Pinnus ponderosa* Laws.) seedling grown under varied atmospheric CO_2 and nitrogen levels. Plant and Soil 189: 275-287.

Satomura T, Nakane K, Horikoshi T 2001 Analysis of fine root net primary productivity of trees using minirhizotron method. Root Res. 21: 741-753. (in Japanese with English abstract)

Satomura T, Fukuzawa K, Horikoshi T 2007 Considerations in the study of tree fine-root turnover with minirhizotrons. Plant Root 1: 34-45.

Vogt KA, Grier CC, Meier CE, Edmonds RL 1982 Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* ecosystems in western Washington. Ecology 63: 370-380.

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