Plant Soil
DOI 10.1007/s11104-011-0953-1

REVIEW ARTICLE

Advancing the use of minirhizotrons in wetlands

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Received: 17 March 2011 / Accepted: 9 August 2011

Abstract
Background Wetlands store a substantial amount of carbon (C) in deep soil organic matter deposits, and play an important role in global fluxes of carbon dioxide and methane. Fine roots (i.e., ephemeral roots that are active in water and nutrient uptake) are recognized as important components of biogeochemical cycles in nutrient-limited wetland ecosystems.

Responsible Editor: Angela Hodge.

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However, quantification of fine-root dynamics in wetlands has generally been limited to destructive approaches, possibly because of methodological difficulties associated with the unique environmental, soil, and plant community characteristics of these systems. Non-destructive minirhizotron technology has rarely been used in wetland ecosystems.

Scope Our goal was to develop a consensus on, and a methodological framework for, the appropriate installation and use of minirhizotron technology in wetland ecosystems. Here, we discuss a number of potential solutions for the challenges associated with the deployment of minirhizotron technology in wetlands, including minirhizotron installation and anchorage, capture and analysis of minirhizotron images, and upscaling of minirhizotron data for analysis of biogeochemical pools and parameterization of land surface models.

Conclusions The appropriate use of minirhizotron technology to examine relatively understudied fine-root dynamics in wetlands will advance our knowledge of ecosystem C and nutrient cycling in these globally important ecosystems.

Keywords Fine roots · Minirhizotron · Wetlands · Peatlands · Methodology

Introduction

Wetland ecosystems store more than one-quarter of global terrestrial soil carbon (C) in deep soil organic matter deposits (Batjes 1996; Bridgham et al. 2006) and play an important role in fluxes of the globally important greenhouse gasses, carbon dioxide and methane (Bridgham et al. 2006). Carbon accumulation in wetlands is the result of an imbalance between production and decomposition that is caused by multiple interacting factors, including anoxic and/or acidic soil conditions, cool temperatures, and colonization by highly recalcitrant plant species, including *Sphagnum* mosses (Gorham 1991). These factors generally limit plant-available nutrients (Bridgham et al. 2001), and wetland vascular plant colonization is restricted to a relatively small number of species that are adapted to nutrient-poor conditions (Bedford et al. 1999; Aerts and Chapin 2000).

The vascular plant species that colonize wetlands can exert strong controls over ecosystem C and nutrient cycling (Neff and Hooper 2002) through adaptations that allow them to survive anoxic soil conditions and efficiently use a limited pool of nutrients (e.g., Vartapetian and Jackson 1997; Aerts and Chapin 2000). For example, ephemeral distal fine roots with a narrow diameter that are responsible for plant nutrient and water uptake (Guo et al. 2008b), play a major role in C and nutrient cycling in nutrient-limited wetlands (e.g., Crow and Wieder 2005). Living fine roots are a substantial component of net primary production and plant nitrogen (N) requirement (Weltzin et al. 2000; Bond-Lamberty et al. 2006). Aerenchymous roots can penetrate anoxic zones and aerate waterlogged soil (Colmer 2003), and root turnover in deeper soil may stimulate the decay of partially decomposed organic matter (Frolking et al. 2001), as well as contribute to organic matter accumulation (e.g., Sullivan et al. 2007).

Despite the clear importance of fine roots, their dynamics in wetlands have only rarely been examined. To date, our limited knowledge of root production in wetland systems has largely been based upon sequential soil coring (e.g., Reader and Stewart 1972; Francez 1995) and root colonization of in-growth cores (e.g., Finér and Laine 2000; Nadelhoffer et al. 2002). While these methods have emphasized the relative importance of the root component in these systems (e.g., Weltzin et al. 2000; Murphy and Moore 2010), they require multiple disturbances and are unable to capture short-term patterns of birth, death, and turnover of ephemeral roots (Vogt et al. 1998).

Minirhizotron technology may improve our understanding of ephemeral roots in wetlands. Minirhizotrons allow non-destructive, repeated monitoring of fine roots over time through the permanent installation of clear tubes in the soil profile into which small video cameras or scanners are inserted at regular intervals (Hendrick and Pregitzer 1996). Minirhizotrons were initially envisioned as a cost-effective and relatively less permanent alternative to large rhizotron observatories used to observe *in situ* root dynamics (Bates 1937). The first minirhizotrons consisted of lamp glasses buried in agricultural fields (Bates 1937), and minirhizotron technology has since been used to quantify fine-root dynamics in a number of upland ecosystems (as reviewed in Hendrick and Pregitzer 1996; Johnson et al. 2001).

Common misconceptions regarding the ineffectiveness of minirhizotrons in wetlands have limited their use, and by extension, have also limited our knowledge of root processes in these systems. Only a handful of studies have employed minirhizotron technology in wetlands (Aerts et al. 1989; Steinke et al. 1996; Steele et al. 1997; Baker et al. 2001; O'Connell et al. 2003; Rodgers et al. 2003; Ruess et al. 2003; Rodgers et al. 2004; Sullivan and Welker 2005; Kalyn and Van Rees 2006; Dickinson 2007; Sullivan et al. 2007; Sullivan et al. 2008; Sloan 2010). While these studies provide evidence that minirhizotron technology can be used effectively to examine fine-root dynamics in these systems, there remains a need for review and standardization of approaches to mange outstanding problems associated with unique wetland characteristics.

Our objective was to develop and communicate a methodological framework for the installation and use of non-destructive minirhizotron technology to examine fine-root dynamics in wetlands. Many of the issues and suggestions in this manuscript are unique to wetlands, whereas others might be of special concern in wetlands but are not unique to those ecosystems. This topic is timely in that it coincides with several new and on-going studies in the United States, Canada, and Scandinavia that use minirhizotrons to examine root dynamics in wetlands (e.g., SPRUCE, Marcell Experimental Forest, Minnesota, USA, http://mnspruce.ornl.gov; PEATcosm, Northern Research Station, Houghton, MI, USA, http://nrs.fs.fed.us/clean_air_water/local-resources/downloads/peatcosm_information.pdf; Mer Bleue Long Term Fertilization Experiment, Ontario, Canada; and ABACUS, Scandinavia, www.abacus-ipy.org).

Wetland characteristics that may affect minirhizotron use

We focus our discussion here on minirhizotron use to quantify relatively understudied root dynamics in wetland ecosystems that have in common at least two characteristics: poor drainage and an accumulation of thick organic horizons. These systems encompass peatlands, boreal forest, arctic tundra, marshes, and swamps. Peatlands are wetlands characterized by soil organic matter deposits at least 40 cm thick (Bridgham et al. 2006). Peat-forming plant species that colonize peatlands (i.e., *Sphagnum* mosses in nutrient-poor bogs and poor fens, graminoid species in rich fens with greater available nutrients, and rainforest trees in tropical peat swamps) contribute to a buildup of soil organic matter that averages greater than 2 m depth globally (Gorham 1991). Poorly-drained ecosystems in boreal forest and arctic tundra also store a substantial amount of soil C in thick organic horizons (i.e., 20 to 30 cm depth; Ruess et al. 2003; Sullivan et al. 2007). Perennially flooded wetlands that have developed on mineral soil, such as marshes and swamps typified by emergent herbaceous vegetation or woody vegetation, respectively (Mitsch and Gosselink 1986), also sequester a significant amount of organic matter in buried sediments (Bridgham et al. 2006).

A variety of root adaptations of vascular species in wetlands could affect vertical root distribution, as well as root morphology and chemistry. For example, black spruce (*Picea mariana* (Mill.) Britton, Sterns & Poggenb.) and tamarack (*Larix laricina* (Du Roi) K. Koch), which are dominant tree species in the boreal forest and in forested bogs and poor fens, tend to restrict their rooting zone to the shallow layer of aerated peat above the water table (Lieffers and Rothwell 1987; Burns and Honkala 1990) and develop adventitious roots (Krause and Morin 2005; E. Kane, *pers. comm.*). The fine roots of ericaceous shrubs, which dominate the understory of nutrient-limited peatlands, are also shallowly rooted (Murphy and Moore 2010), but are distinguished from tree roots by their smaller average diameter (Valenzuela-Estrada et al. 2008). In contrast to woody species, sedges, which are found in the waterlogged northern tundra, across a variety of peatland ecosystems, and in marshes, develop gas exchange root features called aerenchyma that permit rapid transport of oxygen to deep roots (Moog and Janiesch 1990). As a result, sedges are often found deeper in the peat profile than roots of woody plants (Moore et al. 2002; Sullivan et al. 2007; Murphy et al. 2009a), which can allow greater access to soil nutrients (Chapin et al. 1988; Kohzu et al. 2003). Forbs, although relatively rare in nutrient-poor wetlands, can also have aerenchymous root systems (Rydin and Jeglum 2006).

Mycorrhizae play an important role in nutrient-limited wetlands (e.g., Thormann 2006a; Thormann 2006b). Furthermore, potential differences in root lifespan and morphology exist between mycorrhizal and non-mycorrhizal species (Muthukumar et al. 2004), and the association of different types of mycorrhizae with unique root morphologies (Allen et al. 2003) may assist in species-specific root identification in minirhizotron images. For example, all woody, non-ericaceous plant species examined across a gradient of Canadian peatlands were colonized by ectomycorrhizae, while all ericaceous shrubs were found to host ericoid endomycorrhizae (Thormann et al. 1999). In contrast, sedge colonization by arbuscular mycorrhizae may be low in wetlands (e.g., Thormann et al. 1999) due to low soil pH and high soil moisture (Miller et al. 1999). While ericoid and arbuscular mycorrhizal fungi are not readily visible on root tips at the scale of typical minirhizotron images, ectomycorrhizae form sheaths around root tips that are visible in minirhizotron images (e.g., Fig. 1).

Recommendations on the use of minirhizotron methodology in wetland ecosystems

Minirhizotron installation

Minirhizotrons are often installed in upland ecosystems using an angled guide and soil core sampler (as reviewed in Johnson et al. 2001). This system may work well in wetland systems that are poorly-drained but not perpetually flooded (e.g., Ruess et al. 1998; Kalyn and Van Rees 2006; Sullivan et al. 2007). However, alternative methods may be necessary in wetlands where a shallow water table could lead to the collapse of a pre-made hole, low density peat may not support the weight of the guide, or both. For example, in a series of northern peatlands, shallow pilot holes were needed to core through dense root mats in the upper 20 cm of peat, and minirhizotrons

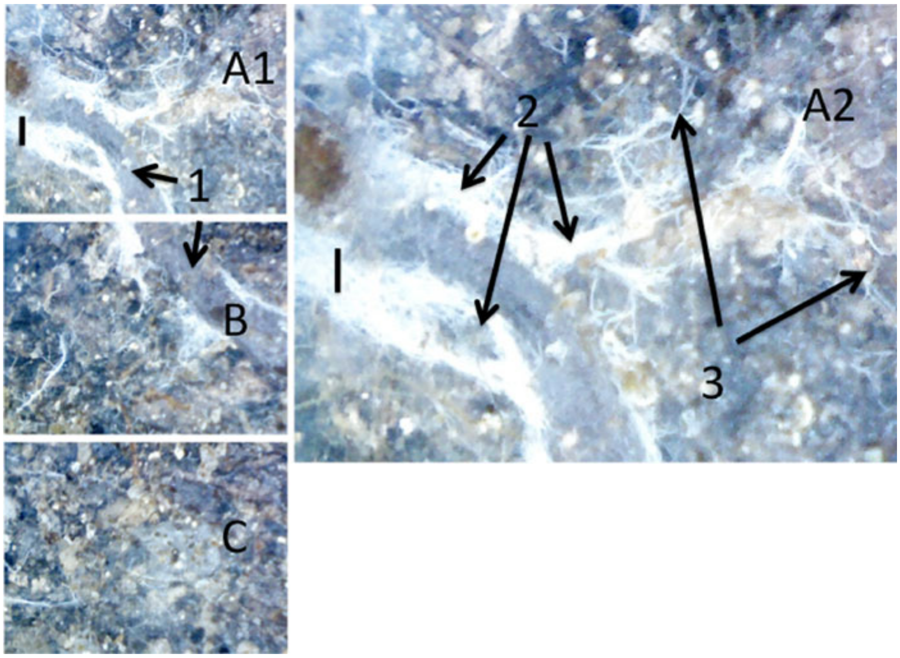


Fig. 1 Minirhizotron image magnification may be an important consideration for the experimental question of interest. *In situ* minirhizotron images were captured with the Automated Mini-Rhizotron (AMR) camera (University of California, Riverside, CA, USA). A digital microscope camera was attached to a movable sled within a sealed minirhizotron tube, and linked via a connecting cable to a computer with software to control sled movement and to collect and transfer images to a server via the internet. Images were collected from under the snowpack in January, 2011 in a mixed conifer forest located at the James Reserve University of California Nature Preserve (Riverside County, California, USA; images from unpublished work by M. Allen). The forest was dominated by *Pinus ponderosa* C. Lawson, *P. lambertiana* Douglas, *Calocedrus decurrens* (Torr.) Florin, *Quercus chrysolepis* Liebm., and *Q. kelloggii* Newberry. RootView software was used to patch together the matrix of individual images. Images A, B, and C are linear individual AMR images showing a fine root with an ectomycorrhizal short root; individual ectomycorrhizal hyphae from A1 can be seen in larger A2 image. Arrow 1 indicates a fine root, arrow 2 indicates an ectomycorrhizal mantle, and arrow 3 indicates rhizomorphs of the ectomycorrhiza. The scale bar is 100 μm in length in images A1 and A2. More AMR images can be viewed at http://ccb.ucr.edu/amarssdata.html

were then pushed or pounded (e.g., with a post-hole driver) into deeper unconsolidated peat material (C. Iversen, M. Murphy, V. Sloan, unpublished). In situations where it is not possible to use a guide, care should be taken to avoid creating too large a hole at the peat surface, as gaps or light leaks could affect estimates of shallow root dynamics (e.g., Hendrick and Pregitzer 1996). Modification of the minirhizotron tube body could further facilitate minirhizotron installation. For example, a machined cone or point fitted to the end of the minirhizotron (e.g., Dickinson 2007), could help prevent large increases in peat density (and therefore pressure) at the bottom of the minirhizotron. It may be useful to coordinate minirhizotron installation with the installation of boardwalk to limit disturbance in sensitive wetland environments and provide access for future minirhizotron image collection.

The method of minirhizotron installation may influence choice of minirhizotron tube material, given that tensile strength varies among different materials (Withington et al. 2003). The potential risks and benefits associated with choosing a specific tube material, which generally includes cellulose acetate butyrate (CAB), acrylic, polycarbonate, and glass, have been reviewed previously (Withington et al. 2003). While both CAB and acrylic minirhizotrons have been successfully installed and used in northern wetlands (e.g., Sullivan et al. 2007; Sloan 2010), acrylic material may be superior to CAB due to the potential effects of tube material on root lifespan (Withington et al. 2003).

Flooding is a concern in ecosystems in which the water table is very close to the soil surface, and we have observed some level of flooding from the bottom of our minirhizotrons (M. Murphy, V. Sloan, P. Sullivan, unpublished). Minirhizotrons should be sealed at the bottom with adhesive and non-toxic marine-grade silicone, and the relatively common slow leaks in these systems may be successfully dealt with before camera insertion using lint-free absorbent cloths and/or a hand vacuum pump system. Frequent cleaning with an absorbent cloth during the imaging process may also help to manage condensation on the inner surface of the minirhizotron tube, which we have found to form quickly in systems with high humidity and a relatively steep temperature differential between the inside and the outside of the tube. Rubber pipe grippers, rubber stoppers, soil sleeves, or PVC end caps, combined with aluminum or electrical tape, may be used as covers to prevent water from coming in through the upper end of the minirhizotrons.

The angle of minirhizotron installation has generally been approximately 45 degrees (as reviewed in Johnson et al. 2001) in order to reduce artifacts associated with vertical installation, such as preferential root growth or water flow along minirhizotrons (Bragg et al. 1983; Johnson et al. 2001). Also, vertically installed minirhizotrons may be unable to capture vertically-growing roots, especially at depth (Bragg et al. 1983). Without the help of an installation guide, the angle of minirhizotron installation in wetlands will likely not be exact (e.g., Ruess et al. 2003; Sloan 2010). A magnetic protractor can be used to facilitate the correct angle of installation, and a few degrees difference can be easily measured and corrected for in subsequent analyses.

It is important to comprehensively sample wetland microtopography with minirhizotron placement, as microtopography is associated with gradients in soil nutrient availability and plant tissue nutrient concentrations (Eppinga et al. 2010), plant community composition (Baird et al. 2009) and root production and rooting depth distributions (Dickinson 2007; Sullivan et al. 2008; Murphy and Moore 2010). Sufficiently large topographic features, including hummock-hollow complexes (e.g., Johnson and Damman 1991), strings (i.e., aggregates of hummocks) and flarks (i.e., aggregates of hollows, Baird et al. 2009) and permafrost features in arctic wetlands such as frost boils (Peterson and Krantz 2008), ice wedge polygons (Minke et al. 2009), and thermokarst (Jorgenson et al. 2001), can be sampled using a stratified random approach. Smaller topographic features, such as tussock production by some sedge species (e.g., *Eriophorum vaginatum* L., Chapin et al. 1979), will likely be sufficiently captured with a completely random sampling design. At a minimum, we recommend making notes of the vegetation composition above and adjacent to each minirhizotron (e.g., Sullivan et al. 2008).

Minirhizotron tubes are the least expensive component of minirhizotron technology (Hendrick and Pregitzer 1996). It is relatively easy and cost-effective to install several minirhizotrons in a given plot (i.e., more than called for in the initial experimental design) as insurance against the extreme environmental conditions in wetlands, and the spatial heterogeneity of root distributions (Jackson and Caldwell 1993). The number of minirhizotrons needed to detect differences in root-length density at a 95% confidence level will depend on the variance among tubes. For example, a minimum of eight minirhizotrons per plot was needed to adequately capture root dynamics in irrigated agricultural fields, but a much greater number was needed in non-irrigated fields (Upchurch 1985; Taylor et al. 1990). Furthermore, additional minirhizotrons may be needed to capture the dynamics of relatively sparse root distributions at depth in the soil (Upchurch 1985), which may be especially important in wetlands where graminoid species are present because their aerenchyma permit deeper root penetration into waterlogged peat than woody species (Murphy et al. 2009a; Sloan 2010).

Minirhizotron anchorage

The long-term stability of minirhizotrons is likely to be a concern in wetland ecosystems due to fluctuating water levels (Baker et al. 2001) and winter frost-heave (O'Connell et al. 2003). Furthermore, minirhizotrons may not be firmly stabilized in organic soils with a low bulk density. These factors make it likely that minirhizotrons installed in wetlands will require some form of anchorage to prevent vertical or rotational movement that could limit the long-term tracking of individual roots.

Previous minirhizotron studies in wetlands have used anchors consisting of wood (Sloan 2010), metal conduit (Baker et al. 2001; O'Connell et al. 2003; Rodgers et al. 2003; Rodgers et al. 2004), or steel rebar (Dickinson 2007) installed to a depth between 1 and 3 m, and attached to the minirhizotron using hose clamps, plastic cable ties, or duct tape. However, in contrast to upland ecosystems, it may be important to consider redox dynamics in these often highly acidic and water-logged environments (i.e., Mitsch and Gosselink 1986); a fiberglass or Teflon-coated stainless steel rod could be used in place of metal conduit in order to avoid metal contamination of the surrounding soil.

Some natural features of wetlands may help to anchor minirhizotrons. In boreal and arctic ecosystems, minirhizotrons have been successfully anchored into the permafrost layer (Ruess et al. 1998; Ruess et al. 2003; Sullivan et al. 2008). Indeed, installing minirhizotrons as deeply as possible in the soil profile in most poorly-drained ecosystems may help to stabilize them, even if imaging of deeper (perpetually flooded) layers is not planned. A previous review indicated several additional options for minirhizotron anchorage in upland ecosystems (Johnson et al. 2001) that would likely also work in wetlands, including methods to improve minirhizotron contact with surrounding soil and modification of the minirhizotron tube body.

An indexing handle (Bartz Technology Corp., Carpinteria, CA, USA) is often used to facilitate the imaging of the same soil location over time. However, several studies in arctic tundra have forgone the indexing handle and instead have used minirhizotrons etched or marked with a continuous series of rectangles smaller than the camera's field of view (Sullivan and Welker 2005; Sullivan et al. 2007; Sullivan et al. 2008). An image of each rectangle was collected by moving the camera without the aid of an indexing handle, and only the area within the rectangle was analyzed. There are two potential advantages to this approach in wetlands: (1) it avoids the process of attaching and removing the cuff associated with the indexing handle, which could result in displacement of a minirhizotron that is not securely anchored, and (2) if a minirhizotron does become displaced, the rectangular area to be analyzed can be redrawn using image analysis software to accommodate the displacement.

Minirhizotron cameras

Advancements in technology making it easier to collect, store, and analyze large numbers of minirhizotron images have been the most important driver of minirhizotron use in a number of natural ecosystems (Hendrick and Pregitzer 1996). Root observation at the minirhizotron tube surface has progressed over time (as reviewed in Upchurch 1985; Taylor et al. 1990) from a mirror and electric light bulb (Bates 1937), to a fiberoptic scope (Waddington 1971), a 35-mm camera (Sanders and Brown 1978), a black and white video camera (Dyer and Brown 1980), and a battery-operated, color video camera (Upchurch and Ritchie 1984). Currently, root dynamics are quantified through the digital capture of color video images on computer, or alternatively, with re-purposed digital scanners. Applying recent advancements in optics to minirhizotron technology will further improve our ability to answer important belowground questions that have remained understudied to-date.

Camera systems that are currently available and commonly used for tracking fine-root dynamics are: the BTC-100× minirhizotron video microscope (Bartz Technology Corporation, Carpinteria, CA, USA), the CI-600 digital root imager (CID Bio-Science, Inc., Camas, WA, USA), and the newly-developed Automated Mini-Rhizotron system (AMR; University of California, Riverside, CA, USA). Each camera system has potential strengths and weaknesses with regard to their use in wetlands. In particular, high camera magnification and image resolution may be needed to capture the production and mortality dynamics of the ephemeral root population in peatland communities dominated by ericaceous shrubs with a narrow root diameter (40 to 75 microns, Valenzuela-Estrada et al. 2008). This will also be true of minirhizotron experiments focused on the dynamics of small-diameter mycorrhizal hyphae.

The BTC-100× video microscope captures analog images of one 18-mm wide column of each minirhizotron in depth increments of ~13.5 mm at a magnification of 15× and image resolution of 470 TV lines. While higher magnification is possible with the BTC-100× (i.e., 100×) that would enable quantification of small-diameter roots and hyphae, the magnified image size is reduced to ~3 mm × 2.1 mm at a set location within the camera's field of view, which could limit examination of root interactions and branching, as well as roots at deeper soil depths where they are relatively scarce.

The CID root imager scans ~345° of the inner surface of a minirhizotron in ~20 cm depth increments; digital images are captured at a maximum resolution of 1200 dpi. However, no magnification is available. Furthermore, high-resolution scanning with the CID system is prohibitively time-consuming and may not be possible in remote wetland sites where battery use is necessary.

The AMR system may facilitate the quantification of small-diameter roots and hyphae in wetlands by improving the size and magnification of minirhizotron images. An automated digital microscope camera in each minirhizotron tube collects individual high-resolution images from a large portion of the minirhizotron surface in increments of 1 mm × 1.2 mm at a magnification of 100× (magnification up to 400× is possible depending on the camera system used) up to four times per day without disturbing the soil surface. Additional benefits of the AMR system in wetlands include a permanently sealed tube, which could help to prevent flooding, as well as automated image collection that could prevent minirhizotron shifting from the disturbance associated with inserting a camera at the surface. However, the purchase of multiple camera systems will be costly, the automated system requires wireless access, and AMR has not yet been tested in wetland ecosystems. Also, wetlands located in extreme climates such as cold boreal and arctic biomes may benefit from a camera system with fewer moving parts.

Frequency of minirhizotron image collection

The timing and frequency of image collection from the minirhizotrons should depend on the question of interest. Event sampling (i.e., intensive filming intervals on the order of hours or days), can be useful for determining root phenology or short-term root population responses to environmental or biological changes. For example, while water-table level often drives important processes in wetlands (Funk et al. 1994), including average rooting depth distribution (Murphy et al. 2009b; Murphy and Moore 2010), we currently have a poor understanding of how quickly roots respond to changes in water-table depth within a growing season. While increased sampling frequency leads to the additional collection of images that are very time-consuming to analyze, Johnson et al. (2001) show that the analysis of every other minirhizotron frame will likely not significantly alter experimental conclusions provided the number of minirhizotrons is sufficient.

In contrast with event-based sampling which may occur only a few times a year, determining annual root production and mortality requires repeated sampling over annual timeframes (Johnson et al. 2001). Timing of image capture in wetlands has ranged from intervals of 1–2 weeks (Sullivan and Welker 2005; Sullivan et al. 2008) to 1 month or more (Aerts et al. 1989; Steinke et al. 1996; Steele et al. 1997; Baker et al. 2001; O'Connell et al. 2003; Rodgers et al. 2003; Rodgers et al. 2004; Sullivan et al. 2007). However, 3 days to 1 week have been previously recommended as the longest sampling intervals to avoid underestimating root production and mortality in upland ecosystems (Stewart and Frank 2008; Kitajima et al. 2010), and there is little evidence to indicate that environmental conditions in wetlands result in substantially slower root turnover when compared with uplands (e.g., Gill and Jackson 2000). Roots may be active during winter months (e.g., Anderson et al. 2003), making it necessary to capture root dynamics outside of the growing season in order to accurately represent total annual root production and mortality. Root overwintering may be especially important in wetland systems where a shallow water table, low bulk density, and snow insulation may limit the depth of winter freeze. However, root observation during winter months will depend on site accessibility and whether the minirhizotron camera can be safely inserted into and removed from the minirhizotron (e.g. frozen soil may compress the minirhizotron tube and hinder camera insertion). All three camera systems discussed here function to at least 0°C (P. Sullivan, V. Sloan, M. Allen, unpublished).

Recommended filming intervals assume that root production and mortality at the minirhizotron surface is in equilibrium with the surrounding soil. However, the length of time it will take for the root population to colonize the minirhizotrons and develop an age structure similar to that in the undisturbed soil is uncertain. While a waiting period of 6 months to 1 year has been recommended for the minirhizotrons to reach quasi-equilibrium (Hendrick and Pregitzer 1996; Johnson et al. 2001), it could take as long as 4 years for root population turnover to stabilize (e.g., Iversen et al. 2008). Unfortunately, to our knowledge there are no long-term minirhizotron data sets from wetlands for comparison (most studies have been conducted for 3 years or less).

Minirhizotron image analysis

There are a number of software packages available for the analysis of minirhizotron images obtained from video or scanning equipment, including RooTracker (Duke University National Phytotron, Durham, NC, USA), WinRHIZO Tron (Regent Instruments, Inc., Quebec, Canada), and Rootfly (Birchfield and Wells, Clemson University, Clemson, SC, USA). More conventional GIS software such as MapInfo (Pitney Bowes Mapinfo Corporation, New York, NY, USA) may also be adapted for root image analysis (e.g., Bai et al. 2010; Sloan 2010). Each software package has strengths and weaknesses with regard to the analysis of fine roots in wetland ecosystems. Some systems allow realignment of the image analysis frame (e.g., RooTracker and GIS software) which is useful in tracking roots in wetlands where minirhizotron movement or peat subsidence may be common. Other software allows the tracking of multiple diameters per root (WinRHIZO Tron), or automated root detection (Rootfly), although the latter may miss small-diameter and older pigmented roots in peatlands (M. Murphy, unpublished).

One of the benefits of minirhizotron technology when compared with soil coring is the ability to track the growth and mortality of individual roots. However, fluctuating water tables, frost-heave, peat shifting, or peat subsidence could lead to the disappearance of roots from a given minirhizotron image frame as a result of processes other than mortality or decay (e.g., Fig. 2), while condensation, ice, or water may obscure images (O'Connell et al. 2003). Image analysis may also be hindered by the fibrous nature of peat formed from decaying *Sphagnum* moss or graminoid material, which does not provide an ideal contrast to differentiate older, pigmented roots (e.g., Fig. 3). Minirhizotron anchorage, combined with the installation of "extra" minirhizotrons may help to alleviate some of these problems, but other problems will require careful consideration of data quality and use (e.g., O'Connell et al. 2003).

Slow root decomposition rates in wetlands due to acidic, water-logged conditions (i.e., Moore et al. 2007) may further complicate the already challenging task of root lifespan analysis. Root mortality may be difficult or impossible to determine because dead roots may remain structurally intact in wetlands for longer periods of time than in uplands. Some researchers have addressed this issue by assigning a tentative mortality designation based on visual characteristics; when combined with continued re-analysis of individual roots, this approach can assist in more accurate mortality estimates (e.g., Rodgers et al. 2003). Though ultraviolet light has been suggested previously as way to differentiate live and dead roots on minirhizotron images (Wang et al. 1995), this technology has not proven reliable (Wang et al. 1995; Baker et al. 2001; Rodgers et al. 2003).

Given potential difficulties associated with tracking the fate of individual roots and determining root

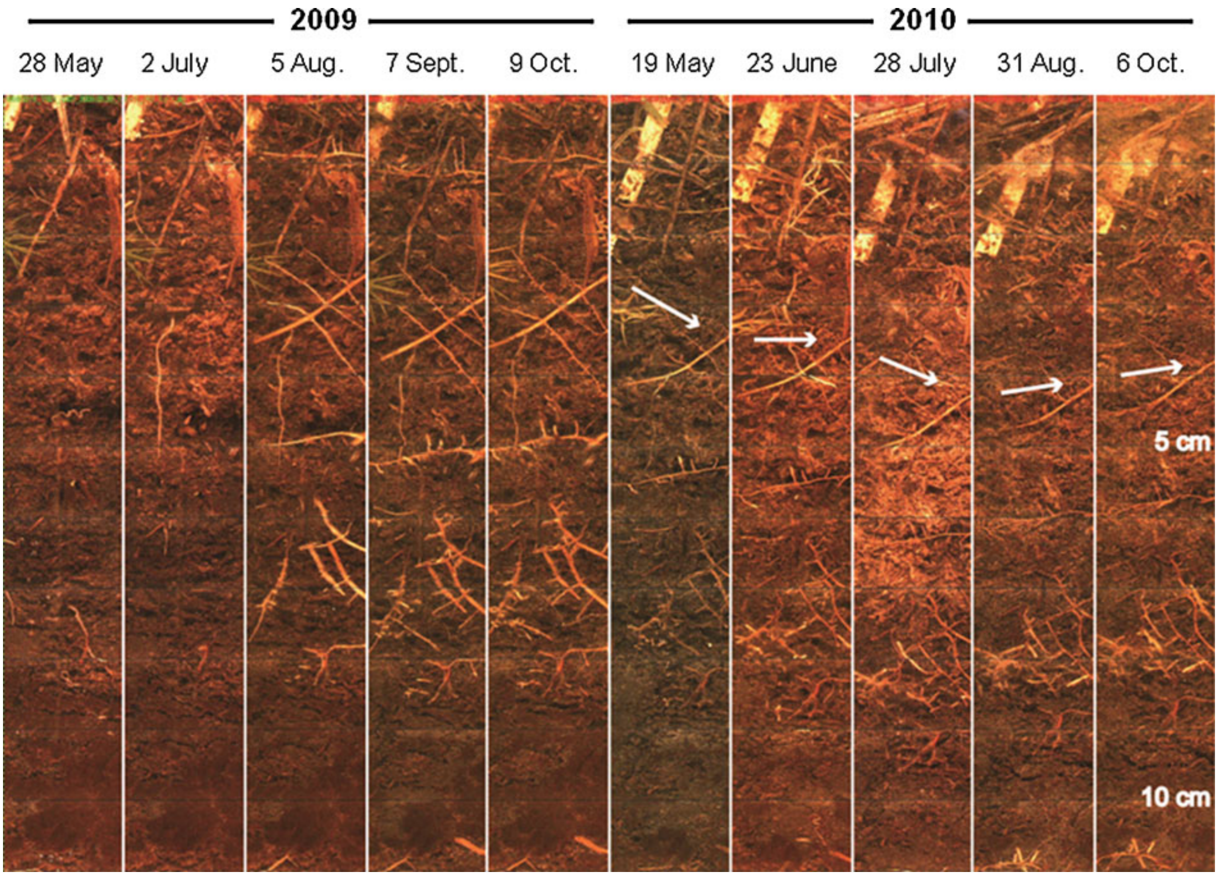
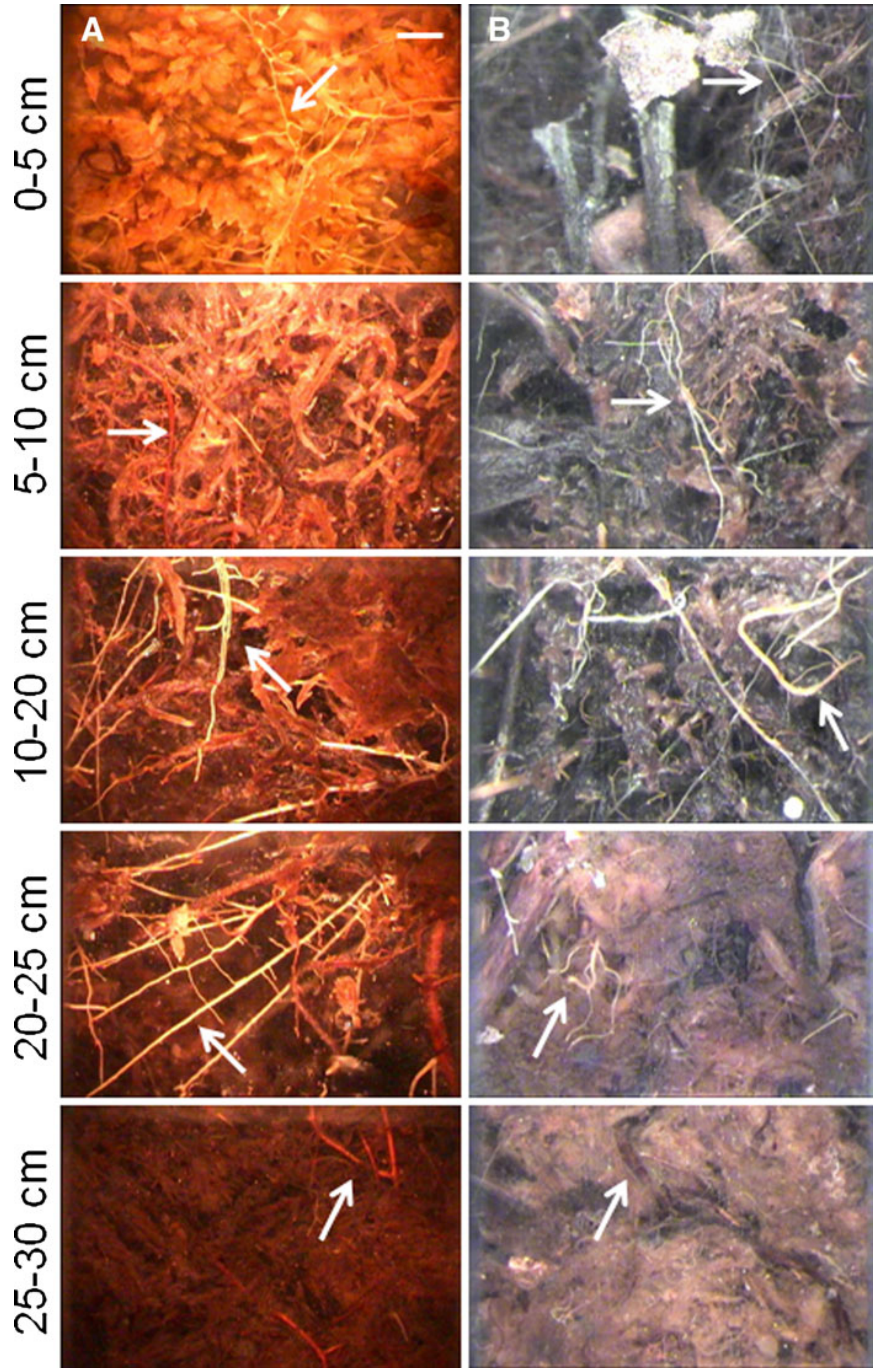


Fig. 2 Seasonal shifting was observed in the surface layer of a *Pinus sylvestris* L. forest peatland in southern Finland that was drained in 1970. Minirhizotron images were collected with the BTC-100× minirhizotron video microscope (Bartz Technology Corporation, Carpinteria, CA, USA), and were pieced together in vertical columns representing the peat profile on each of ten sampling dates from May, 2009 to June, 2010 (images from unpublished work by T. Sarjala). Images are shown here to a depth of ~11 cm. As indicated by the appearance of the same roots in different minirhizotron images over time, peat initially shifted downward between image collection dates in October, 2009 and May, 2010 (i.e., over winter). Peat continued to subside throughout the summer of 2010, but began to shift upward on the last two sampling dates of 2010 (see arrows)

mortality in wetlands, root population turnover may be calculated instead as total annual fine-root production or mortality divided by peak fine-root standing crop (Gill and Jackson 2000). However, this method must be used with caution, as it assumes that the root population has fully colonized the minirhizotrons, which could take years (Iversen et al. 2008) and also assumes that root production and mortality are in equilibrium. Furthermore, living root biomass at peak standing crop may be a relatively small percentage of total root biomass (living and dead) in wetlands (i.e., less than 20%, Bernard and Fiala 1986) due to slow root decomposition rates. Unfortunately, no easy correction factor exists, as current methodologies to differentiate living and dead roots in soil cores can be somewhat subjective (e.g., root coloration and tensile strength, Aerts et al. 1989) or inconclusive (e.g., staining to determine metabolic activity, Sturite et al. 2005).

Differences among functional groups in their rooting dynamics and chemistry may also necessitate additional analytical considerations. Whenever possible, roots should be differentiated to at least a functional life-form designation (i.e., tree, shrub, sedge); this may be aided by unique morphological features, such as diameter distribution, coloring (e.g., relatively pale sedge roots compared with pigmented woody roots), growth habit (e.g., highly-branched ericaceous roots compared with sparsely-branched and vertically-rooted sedges), or mycorrhizal colonization.

Given the known differences in the amount of root geotropism among species and root types, it is



important to consider species-specific interaction with the minirhizotron surface (e.g., Sorrell et al. 2000). Three plant communities in northern Finland (Sloan 2010) were found to differentially colonize the upper, side, and bottom surface of minirhizotrons (Fig. 4). The vertical growth habit of sedge *Eriophorum angustifolium* Honck. resulted in up to ~50% of total annual root length being produced along the upper-

◀ **Fig. 3** Minirhizotron images indicate some common peat and fine-root characteristics of bogs. Images were captured from two different bogs in October, 2010 using the BTC-100× minirhizotron video microscope system (Bartz Technology Corporation, Carpinteria, CA, USA). The peatlands are: (a) a black spruce bog at Marcell Experimental Forest, northern Minnesota, USA (SPRUCE, http://mnspruce.ornl.gov/; images from unpublished work by C. Iversen and J. Childs), and (b) an open bog at Mer Bleue Conservation Area in Ontario, Canada (Mer Bleue Long Term Fertilization Experiment; images from unpublished work by M. Murphy). Images shown are from one minirhizotron located in a hummock at each site; the depth range from which each image was captured is located at the left. Scale bar is 2 mm in length; arrows in each image indicate a fine root (most images contain multiple fine roots). While new (i.e., white) fine roots show up well against the peat matrix consisting mainly of decomposing *Sphagnum* mosses, older, more pigmented roots are difficult to observe (e.g., 5–10 cm depth in column A, and 25–30 cm depth in columns A and B). Furthermore, loosely consolidated peat may create large gaps or pockets that may make it difficult to determine a depth of field, and also to decide which roots should be included in the analysis of a given image (e.g., 10–25 cm depths in column A, 0 to 20 cm depths in column B). Some images are obviously underwater (e.g., 25–30 cm depth in column A, and 20–30 cm depth in column B); a qualitative analysis of image water content could potentially be used as a covariate in root analyses. See also Dickenson (2007) for images of roots in a flooded, freshwater swamp in southeastern Virginia, USA

facing quadrant of the minirhizotron surface for at least three years after minirhizotron installation (Fig. 4a). This underscores the need for angled minirhizotron installation to capture root dynamics in systems dominated by vertically-rooted sedges. However, these data also indicate that care must be taken in the interpretation and upscaling of root data from minirhizotron images in systems colonized by vertically-rooted species, as data extrapolated from the upper minirhizotron surface could greatly overestimate the density of geotropic roots. In contrast, the non-differential surface colonization of minirhizotrons in systems dominated by woody species (Fig. 4c) might enable the analysis of minirhizotron surfaces other than the top surface in these systems to avoid the potential effects of gaps, light leaks, and soil drying on the quantification of root dynamics at the peat surface (e.g., Johnson et al. 2001).

Scaling root length and diameter to biogeochemical pools

Scaling minirhizotron data from idiosyncratic units (i.e., root length per frame or per minirhizotron surface area) to units comparable to aboveground production is necessary to determine the contribution of the fine-root population to ecosystem processes (Johnson et al. 2001). Scaling involves two steps: (1) scaling from minirhizotron surface area to soil volume, and (2) scaling from root length and width to root mass. A depth-of-field approach, where it is assumed that the roots observed in the minirhizotron frame fill some volume of soil, has often been used to upscale minirhizotron data from root length per minirhizotron surface area to root length per soil volume. A 3-mm depth of field was initially used to scale root length (Sanders and Brown 1978), but in practice, the assumed depth of field has ranged from 2 mm to 3 mm in upland systems (Johnson et al. 2001) and in wetlands. For example, a 3 mm value was chosen in sedge-dominated arctic tundra (Sullivan and Welker 2005; Sullivan et al. 2007; Sloan 2010) based on comparison of minirhizotron data with values in the literature (Sullivan and Welker 2005) and estimates of annual production made using in-growth cores (Sullivan et al. 2008), whereas a 2-mm depth of field was used in poorly-drained boreal black spruce forests (Steele et al. 1997; Ruess et al. 2003). Loosely consolidated peat in wetland ecosystems may make it difficult to estimate an average field depth, especially at shallow soil depths in where bulk density is least (Rodgers et al. 2004; Fig. 3, this paper). As the assumed depth of field can drastically affect root production estimates (e.g., Metcalfe et al. 2007), objective methods for defining depth of field (e.g. from automated analysis of image edge resolution), and for selecting roots to include in a given analysis (e.g., derived from root-edge sharpness), seem both possible and desirable.

While the depth-of-field approach allows an estimate of root length per unit soil volume, root length must be converted to biomass units in order to inform ecosystem C and nutrient budgets. Diameter is a useful metric for scaling minirhizotron data to root biomass and nutrient content, as root width is relatively easily observed in high-resolution minirhizotron images. Most studies use the specific root length (SRL) of one or more diameter classes of root determined from soil cores to scale to root biomass from minirhizotron data (Johnson et al. 2001). However, the application of a single value of SRL to a broad diameter class (e.g., 0 to 1 mm as in Johnson

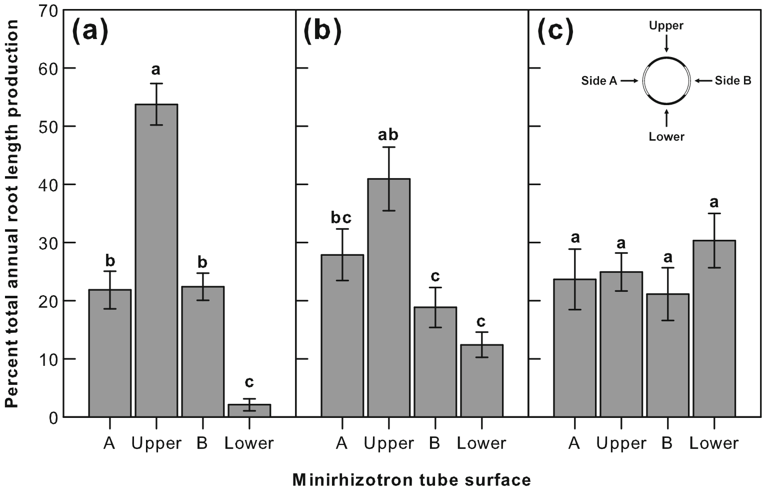


Fig. 4 Percent distribution of total annual root length production around the minirhizotron surface determined from ~360° scans (CI-600 digital root imager, CID Bio-Science, Inc., Camas, WA, USA) taken in three plant communities in northern Finland in 2008 (Sloan 2010). A cross-section of a minirhizotron tube is provided in panel c to indicate how the minirhizotron surface was divided for this analysis; each section represented one quarter of the tube. Data are mean±1 SE ($n=8$ minirhizotrons in each plant community). Within each plant community, a one-way ANOVA followed by Tukey's HSD test were used to determine differences in annual root length production among each quarter of the minirhizotron tube surface. The three communities examined were dominated (>10% cover) by different species and comprised: (a) a sedge community in standing water, dominated by *Eriophorum angustifolium* Honck. and *Carex chordorrhiza* Ehrh. ex L. f., (b) a forest margin dwarf shrub community on well-drained organic soil, dominated by *Empetrum nigrum* L., *Vaccinium uliginosum* L., and *Calluna vulgaris* (L.) Hull, and (c) a forest community on well-drained organic soil, dominated by *Betula pubescens* ssp. *tortuosa* (Ledeb.) Nyman, *Betula nana* L., *E. nigrum*, and *Vaccinium vitis-idaea* L. Within each community, different letters indicate significant differences in annual root length production among quadrants of the minirhizotron surface ($P<0.05$), while shared letters indicate no significant difference (e.g., side "A" and the "Upper" surface in panel b). Differences in minirhizotron surface colonization were likely because of a difference in rooting habit of the dominant species in each community. Sedges were vertically-rooted, while some shrubs in the forest edge community rooted from shallow rhizomes, and *B. pubescens ssp. tortuosa* in the forest community was horizontally-rooted. Across all three sites, it appears that minirhizotron sides may be the most representative of total tube colonization (~25% total annual root length production at each site)

et al. 2001) can substantially overestimate the weight of smaller-diameter roots. Continuous relationships between root diameter, and root biomass, N concentration, or respiration exist for a number of species (Iversen et al. 2008; Makita et al. 2009), including those in wetlands (Sullivan et al. 2007).

Given the difficulties in determining a depth of field from minirhizotron images taken in wetlands, and the fact that minirhizotrons may not intercept roots in the soil in a way that represents the average rooting distribution, an alternative approach may be needed to determine root biomass production or mortality. One alternative may be to use soil cores to determine root standing biomass, which is then scaled to production or mortality using length-based annual root turnover rates (or root lifespan estimates) derived from minirhizotron images (Hendrick and Pregitzer 1996). As different root diameter classes, as well as different species, will likely have different turnover rates, the scaling can be done separately for each root type to obtain an average ecosystem-level estimate of root biomass production or mortality. However, this method may also be challenging in wetlands, given issues with (1) estimating root turnover from minirhizotron images, and (2) obtaining accurate estimates of the biomass of small-diameter ericaceous roots from soil cores (e.g., Murphy et al. 2009b).

Up-scaled estimates of root biomass production from minirhizotron data have been assessed in wetlands using in-growth cores installed at an angle to mimic the minirhizotrons and to better capture the dynamics of vertically growing root populations (e.g., Sullivan et al. 2007; Sullivan et al. 2008; Sloan 2010). One benefit of using in-growth cores in wetlands is that it may be easier to separate out small-diameter ericaceous shrub roots from milled peat rather than from an intact peat matrix (i.e., Murphy and Moore 2010), though very fine roots may still be lost (Finér and Laine 2000). However, in-growth cores have a number of problems (Vogt et al. 1998), which include long sampling intervals that could miss a substantial fraction of root production and mortality, as well as a higher proportion of pioneer roots that are destined to be structural rather than nutrient-absorbing (Zadworny and Eissenstat 2011). Additionally, while root production estimates from in-growth cores have been found comparable with minirhizotron production estimates in some wetlands (Sullivan et al. 2008), in other wetlands, in-growth cores were poorly colonized by roots (Sloan 2010).

It is well known that the ontological order of root production (i.e., root order) is an important driver of root physiology, nutrient content, turnover, and mycorrhizal colonization (Pregitzer et al. 2002; Guo et al. 2008b; Valenzuela-Estrada et al. 2008). However, root order is less easily observed than root diameter in smaller minirhizotron images, so camera systems with larger frames of analysis may be necessary for root order analyses. Alternatively, intensive surveys of root anatomy conducted on intact root systems may help to determine the diameter range of ephemeral roots before the minirhizotron study is undertaken (e.g., Pregitzer et al. 2002; Guo et al. 2008b; Valenzuela-Estrada et al. 2008). In woody plants, ephemeral roots (i.e., roots with a primary function of resource uptake) are those roots that are non-woody and have an intact cortex (Guo et al. 2008b). The number of lateral root branches (i.e., orders) that comprise ephemeral roots, as well as the diameter of different root orders, may differ among species (e.g. Pregitzer et al. 2002; Guo et al. 2008b). After relationships between root morphology and physiology have been determined, a species-specific average diameter may be used to determine root order from minirhizotron images (Valenzuela-Estrada et al. 2008). In so far as ephemeral roots can be clearly separated from longer-lived, woody roots based on root diameter (which may not be possible in all instances, e.g., Guo et al. 2008b), the order-diameter relationship will allow subsequent minirhizotron analyses to focus on ephemeral root dynamics.

The minirhizotron technique is most useful for understanding the contribution of ephemeral roots (i.e., the lowest root orders) to ecosystem C and nutrient budgets because small observation windows may not capture the dynamics of higher-order roots. Also, woody roots with a slower growth rate may take longer to colonize the minirhizotron tubes (e.g., Pritchard and Strand 2008). Therefore, estimates of root C and nutrient content extrapolated from minirhizotron data should not be considered representative of all roots in the system. Other methodology, including stable C isotopes, may be useful in this regard (e.g., Guo et al. 2008a; Strand et al. 2008).

Root dynamics should be reported both in terms of absolute depth in the soil, as well as distance to the water table (e.g., Moore et al. 2002). This may be particularly important in the context of understanding the interaction between root dynamics and peat microtopography, as hummocks and hollows are by definition different heights above the water table.

Informing ecosystem and land surface models with fine-root dynamics

Dynamics of fine roots are not currently well-represented in most ecosystem and land surface models (Jackson et al. 2000; Iversen 2010). The unique features of wetlands may further complicate data-model linkages, especially given the poor representation of wetland ecosystems in existing models (Limpens et al. 2008). Challenges in confronting models with fine-root data are related to: (1) predicting continuous root growth and turnover dynamics across ecosystems, (2) understanding how root physiological processes change with rooting depth, (3) understanding root physiological activity outside of the growing season, and (4) correctly representing fractional root allocation and rooting depth distributions across plant functional types. In particular, the depth at which fine roots are produced has important implications for ecosystem C storage in wetlands (Frolking et al. 2001).

While lingering questions remain regarding fine-root dynamics in relatively understudied wetland ecosystems, recent research has uncovered some important predictive relationships between rooting dynamics and relatively easily measured environmental or plant community variables in these systems. For example, Murphy and Moore (2010) found that rooting depth distribution in an ombrotrophic bog in Canada was well-predicted by both average growing season water table depth and aboveground biomass.

Future research should focus on overcoming the difficulty of identifying roots of different species in minirhizotron images to allow the determination of species-specific rooting dynamics and depth distribution. An additional focus should be the examination of root physiology (i.e., root lifespan, nutrient uptake) outside of the growing season.

Conclusions

Minirhizotron technology, despite its imperfections, remains one of the best methods to address questions regarding the dynamics of ephemeral roots in natural ecosystems. However, the deployment of minirhizotron technology in wetlands may be challenged by unique environmental, soil, and plant community characteristics. We have discussed here a number of recommendations for minirhizotron deployment in wetlands with regard to installation and image analysis, and the upscaling of minirhizotron data to inform biogeochemical pools and land surface models. Advancing the use of minirhizotron technology to examine relatively understudied fine-root dynamics in wetlands is important for expanding our knowledge of ecosystem C and nutrient cycling in these systems.

Acknowledgements This paper was developed from a workshop held at Oak Ridge National Laboratory in October, 2010 to facilitate discussion on minirhizotron use to examine fine-root dynamics in wetlands. The authors do not have a financial interest in the commercial products discussed here. We thank the New Phytologist Trust and the U.S. Department of Energy, Office of Science, Biological and Environmental Research for sponsoring the workshop. We also thank R. Norby and anonymous reviewers for comments that improved the manuscript. Oak Ridge National Laboratory is managed by UT-Battelle, LLC for the United States Department of Energy under contract DE-AC05-00OR22725.

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