Commentary

Weighty issues in respiratory metabolism: intriguing carbon isotope signals from roots and leaves

Nonphotosynthetic cells of higher plants depend on the supply of a carbon substrate, usually sucrose, for cellular metabolism. Typically this sucrose is synthesized in leaves from the products of photosynthesis. Because of the lack of light below ground, roots do not generally photosynthesize (we note the exception of certain mangrove species and members of the Orchidaceae), although many roots from genetically distinct taxa, such as Asteraceae, Solanaceae and Cucurbitaceae, have the ability to turn green when grown in the light (Flores *et al.*, 1993). Nonetheless, plant roots generally grow underground as heterotrophic organs, depending on leaves for their energy source.

'... the metabolic origins of respired CO_2 differed not only between heterotrophic and autotrophic phases of development at the whole-plant level, but more interestingly between leaves and roots on the same plant'

There are also periods in the life cycle of all plants when autotrophic inputs are momentarily suspended and the metabolic demand for carbon substrate is supplemented through the remobilization of stored carbohydrate reserves, such as starch. This state is often referred to as heterotrophy, and occurs in all plants to some extent on a diurnal timescale, during the dark; a seasonal timescale, during the springtime spectacle of budburst in deciduous plants; or during the germination and growth of plants from seeds and tubers. Novel and intriguing insights into the transitions between heterotrophic and autotrophic carbon metabolism in whole plants and individual organs are presented by Bathellier *et al.* in this issue of *New Phytologist* (pp. 406–418). They methodically tracked the metabolic transition of French bean development from the initial heterotrophic phase of seed imbibition and germination right through to the expansion of actively photosynthesizing leaves, using a combination of techniques, including the stable carbon isotope ratios ($^{13}C/^{12}C$ or $\delta^{13}C$ when expressed relative to a standard) observed in plant organic matter, metabolites and the respired CO_2 of different plant organs. The $\delta^{13}C$ signals they observed in their work revealed that the metabolic origins of respired CO_2 differed not only between heterotrophic and autotrophic phases of development at the whole-plant level (see their Fig. 3), but more interestingly between leaves and roots on the same plant (see their Fig. 4).

Why are the $\delta^{13}C$ signals of respired CO_2 different between roots and leaves?

Many carbon isotope studies have shown that the carbon isotope composition of CO_2 respired by leaves in the dark is ^{13}C-enriched relative to the potential metabolic substrates of respiration: glucose, sucrose or fructose (see review of Ghashghaie *et al.*, 2003). This is because glucose has a reproducible nonstatistical ^{13}C-pattern, consisting of relatively ^{13}C-enriched carbons in positions C-3 and C-4 of the molecule (Rossman *et al.*, 1991; Fig. 1) that contribute to the release of enriched CO_2 during subsequent decarboxylation of pyruvate by the pyruvate dehydrogenase complex (PDC) (Fig. 2). Although this pathway is also active in roots (Dieuaide-Noubhani *et al.*, 1995), it does not seem to impact the $\delta^{13}C$ of root respired CO_2 to the same extent (Badeck *et al.*, 2005; Bathellier *et al.*, this issue). Other decarboxylations, for instance those from the oxidative stage of the pentose phosphate pathway (PPP) in root plastids, have been shown to contribute large amounts of CO_2 to the total flux from respiring maize root tips (up to 25%) (Dieuaide-Noubhani *et al.*, 1995). This particular decarboxylation is known to occur on the C-1 position of

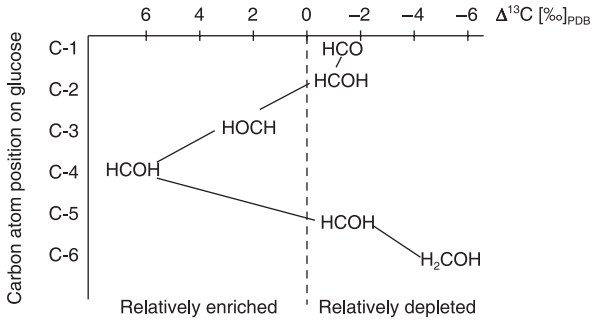

Fig. 1 The relative intramolecular distribution of carbon isotopes in glucose from C3 and C4 plants. Values are deviations from the mean isotope composition $\Delta^{13}C$ [‰] for each carbon position on glucose (adapted from Rossman *et al.*, 1991).

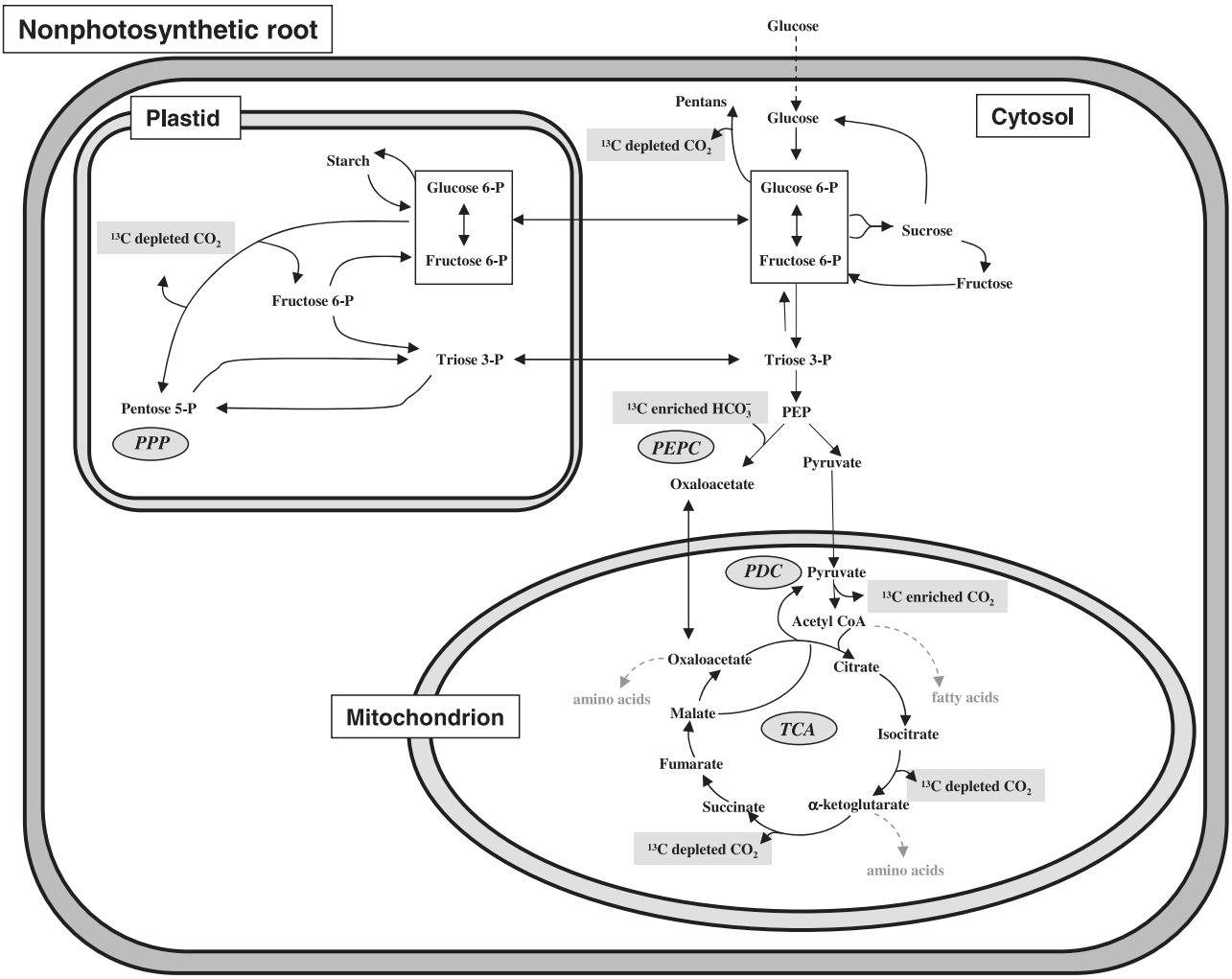

Fig. 2 Scheme showing the potential carboxylation and decarboxylation reactions contributing to the carbon isotope composition of the net CO_2 flux from a nonphotosynthetic root. PPP, pentose phosphate pathway; PEPC, phosphoenolpyruvate carboxylase reaction; PDC, pyruvate dehydrogenation complex; TCA, tricarboxylic acid cycle.

glucose that is always relatively depleted in ^{13}C, thus resulting in a release of ^{13}C-depleted CO_2 (Figs 1, 2). A further contribution of depleted CO_2 could also occur during pentan synthesis, a process also hypothesized to be active in the cytosol of growing roots (*c.* 17% of total flux; Dieuaide-Noubhani *et al.*, 1995) (Fig. 2). Again this particular decarboxylation would lead to the release of depleted carbon from position C-6 of glucose (Fig. 1). Either of the above carboxylations combined with any of the further potentially depleting pathways for CO_2 in the tricarboxylic acid (TCA) cycle (or Krebs cycle) could cause the carbon isotope composition of the net CO_2 flux from roots to be more depleted than those signals observed from leaves. Therefore it seems probable from the growing evidence in the above work that decarboxylating pathways other than the PDC reaction, with distinct and opposite isotopic consequence for the δ^{13}C of root-respired CO_2, must contribute more to the total flux of CO_2 leaving growing roots.

Anaplerotic uptake of CO_2, also known as dark CO_2 fixation, is another metabolic candidate for the release of ^{13}C-depleted CO_2. It is a process necessary in plant foliage, roots and soil microorganisms, especially during periods of active growth and nitrogen assimilation when a demand for the synthesis of carbon skeletons by amino acid synthesis is high (Raven & Farquhar, 1990). To fulfil this demand, carbon intermediates (e.g. citrate) are removed from the TCA cycle for this biosynthesis (Fig. 2). In order to keep this cycle ticking over efficiently, carboxylating enzymes such as phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31), and pyruvate carboxylase (PC, EC 6.4.1.1) synthesize oxaloacetate, thereby replenishing the cycle with carbon intermediates. An inorganic carbon supply is essential for this cycling and can be sourced either through the scavenging of intracellular respired CO_2 or the uptake of CO_2 from the surrounding atmosphere (or soil air pores in the case of plant roots). The rate of anaplerotic CO_2 fixation is difficult to separate from the respiratory efflux

using net CO_2 flux measurements alone. However, because PEPC fractionates by 5.7‰ in favour of ^{13}C, the carbon assimilated into root organic matter should become enriched relative to its inorganic CO_2 substrate, thus causing the remaining CO_2 measured in the soil atmosphere to become more depleted. These isotopic fingerprints of PEPC, and the results from a thorough mass balance, led Bathellier *et al.* to hypothesize that PEPC activity could be responsible for the ^{13}C enrichment observed in organic matter in whole plants (see their Fig. 3) and the corresponding ^{13}C-depleted CO_2 signals during the heterotrophic growth phase.

Impact of divergent isotopic signals at larger scales

The above metabolic processes could also help explain the diurnal and seasonal variability in the $\delta^{13}C$ signals measured at the ecosystem scale. For example, in a Sitka spruce forest, depleted values as low as –32‰ were observed from the soil (Wingate, unpublished), whilst $\delta^{13}C$ signals from respiring whole branches in the dark were more enriched (around –26‰ to –28‰; Wingate *et al.*, 2007). However, these differences in the carbon isotopic signatures from leaves and soils in the dark were observed during canopy budburst in spring only. At other times of the growing season, the $\delta^{13}C$ of soil-respired CO_2 was not as depleted and similar to the $\delta^{13}C$ of bulk soil organic matter, around –28‰. Some other processes linked to important transitions in canopy carbon allocation might also be responsible for these observations. More studies on the phenology of $\delta^{13}C$ signals using chamber measurements on branches, woody stems and soils should allow us to identify when and where metabolic switches occur in forest ecosystems and whether these are linked to transitions in heterotrophic/autotrophic status of the trees. Studying the intra-annual isotopic signals archived in root and stem cellulose should also prove useful in confirming some of these hypotheses, as these high-resolution isotope signals in tree rings imprint such metabolic switches dramatically during the transitions between heterotrophic and autotrophic activity, during budburst and leaf senescence of deciduous trees (Helle & Schleser, 2004).

The work of Bathellier *et al.* brings some timely, highly novel insights into the respiratory isotopic divergence observed between plant organs and stages of development. Such an understanding is needed to model dark respiration and the associated carbon isotopic signals more mechanistically and help us interpret intriguing isotope signals observed at larger scales caused by plant respiration.

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Key words: carbon isotopes, heterotrophy, leaf respiration, phenology, root respiration.

Can you believe what you see? Reconciling minirhizotron and isotopically derived estimates of fine root longevity

Despite their small size, fine roots (i.e. < 2 mm in diameter) have a disproportionate importance in uptake of soil resources. By virtue of their intimate and extensive contact with soil organisms, they link plant metabolism to soil nutrient cycles. Fine roots are ephemeral and must be frequently replaced. Development of new fine roots to replace dying or dead ones (a process called root turnover) is metabolically expensive and may account for 30% of global terrestrial net primary production (NPP) (Jackson *et al.*, 1997). Their

role in the soil environment often draws comparisons with the role of leaves in the aerial environment (Tjoelker *et al.*, 2005). Yet, we know little about basic fine root biology, including how long they live. It is exciting, therefore, to hear about the development of a method for quantifying longevity of fine roots using carbon isotopes. On pages 443–456 of this issue, Guo *et al.* review this new approach and develop a statistical model that explains why estimates of root longevity derived using this new isotope approach do not match previous estimates derived from direct root observations from minirhizotrons.

'If fine roots live many years, as these studies suggest, then the proportion of NPP allocated to support fine root turnover must be substantially less than previously thought'

Choose your method

The most popular approaches for quantifying fine root turnover include sequential soil coring, mass balance approaches, such as the N budget and C budget techniques, and minirhizotrons. There are four variations on the sequential soil coring approach (reviewed in Vogt *et al.*, 1998). These include the max-min method in which production is assumed to equal the difference between the maximum and minimum standing crop observed within a given year. In a related approach, the differences in fine root biomass between sequential harvests are summed throughout the year to estimate production. The third method, referred to as the compartment flow model, is similar but it also incorporates changes in dead root pools and compensates for loss of dead roots by explicitly accounting for decomposition. The last of the traditional soil coring methods is based on sequential coring of root free ingrowth cores. Nitrogen and carbon budget approaches are less direct, and less popular than sequential soil coring methods. The N budget technique estimates fine root production from N mineralization rates and the C budget approaches require accurate inventories of C fluxes at the ecosystem scale, which can be challenging and expensive to obtain (Vogt *et al.*, 1998; Nadelhoffer, 2000). All of these methods have their own advantages, but all are subject to large errors because they rely on multiple tenuous assumptions (Vogt *et al.*, 1998; Nadelhoffer, 2000).

Both soil core methods and mass balance approaches yield indirect estimates of fine root production; neither directly measures longevity of the individual roots. Fine root longevity is equal to the reciprocal of root turnover. Root turnover is calculated by dividing either annual below-ground production or mortality by either maximum or mean yearly fine root standing crop (Gill & Jackson, 2000; Norby and Jackson, 2000). By the time production and standing crop are measured by the approaches described above, and fine root longevity is calculated, considerable error can result even when sampling the same experimental plots (Hendricks *et al.*, 2005).

In recent years, the minirhizotron has become the favorite method for characterizing fine root lifespan because it directly measures individual root longevity from repeated video or digital images (Vogt *et al.*, 1998; Majdi *et al.*, 2005). Recent advances in automating the analysis of minirhizotron images, reported in this issue by Zeng *et al.* (pp. 549–557), could lead to increased use of this method of root analysis. Based largely on minirhizotron experiments, it is now widely accepted that fine roots live an average of 1 yr or less (Guo *et al.*, this issue). Can we believe what we see? Recent estimates of longevity derived from carbon isotopic techniques suggest we cannot.

The controversy

Several recent studies based on changes in carbon isotopic ratios through time, such as bomb ^{14}C techniques, have reported that turnover time of fine roots is between 4.2 and 32 yr (Gaudinski *et al.*, 2001; Tierney & Fahey, 2002; Johnson *et al.*, 2005; Trumbore *et al.*, 2006). In one recent report based on isotope tracer experiments at Duke University and Oak Ridge National Laboratory (ORNL) free air CO_2-enrichment sites (FACE), mean residence time (MRT) of fine root carbon, also referred to as fine root turnover (Trumbore & Gaudinski, 2003), was estimated to be 1.3–3 yr (deciduous forest) and 4.2–5.7 yr (pine forest) (Matamala *et al.*, 2003). These estimates were made possible by fumigating large forest plots with CO_2 possessing a carbon isotopic signature distinct from ambient CO_2. Sequential cores were then harvested and the rate of depletion of old carbon in fine root biomass pools was determined. The rate of disappearance of old carbon was fitted to an exponential decay model to calculate the mean residence time of fine roots.

If fine roots live many years, as these studies suggest, then the proportion of NPP allocated to support fine root turnover must be substantially less than previously thought. The implications of this finding for how we think about root structure and function, soil microbiology, tree nutrition, and carbon cycling are significant. How can such disparate estimates of fine root longevity be reconciled?

The reconciliation

Guo *et al.* developed a statistical model to explore the potential effects of fine root heterogeneity, turnover calculation methods, longevity distribution models, and sampling bias on root longevity estimates. Their model simulations indicated

that median longevity estimates, as commonly obtained with minirhizotrons, always underestimated actual longevity, whereas simulated mean residence time of carbon, as would be obtained from isotopic studies, always overestimated longevity. Their results suggest that heterogeneity in the fine root pool accounts for the majority of this error.

Longevity distributions of fine roots are typically positively skewed, indicating the presence of multiple root pools with inherently different longevities (Fig. 1; Tierney & Fahey, 2002; Trumbore *et al.*, 2006; Joslin *et al.*, 2006). The model of Guo *et al.* accounts for this heterogeneity by assuming that the most dynamic pool is dominated by the smallest first- and second-order roots, while the longer-lived root pool is dominated by larger fourth- and fifth-order roots (Eissenstat *et al.*, 2000; Wells *et al.*, 2002; Guo *et al.*, 2004). Because isotopic studies are based on residence time of mass, they are biased by the larger, fourth- and fifth-order fine roots that are less numerous but contain more carbon and live longer than first- and second-order roots. On the other hand, because minirhizotron estimates are number (not mass)-based, they are biased by the first- and second-order roots, which are most numerous, turn over the fastest, but contain less carbon than higher-order roots. Our data from an 8 yr minirhizotron study at the Duke FACE site are consistent with results obtained by Guo *et al.* We observed a positively skewed longevity distribution for fine roots at the Duke FACE site, indicating the presence of both short- and long-lived fine roots (Fig. 1). We also observed considerable heterogeneity that could not be explained by depth or diameter (Fig. 2) and suggest that branch order might account for much of this variation.

Sampling bias inherent to different methods might also contribute to divergence of minirhizotron and isotopic results, although the modeling study by Guo *et al.* suggests

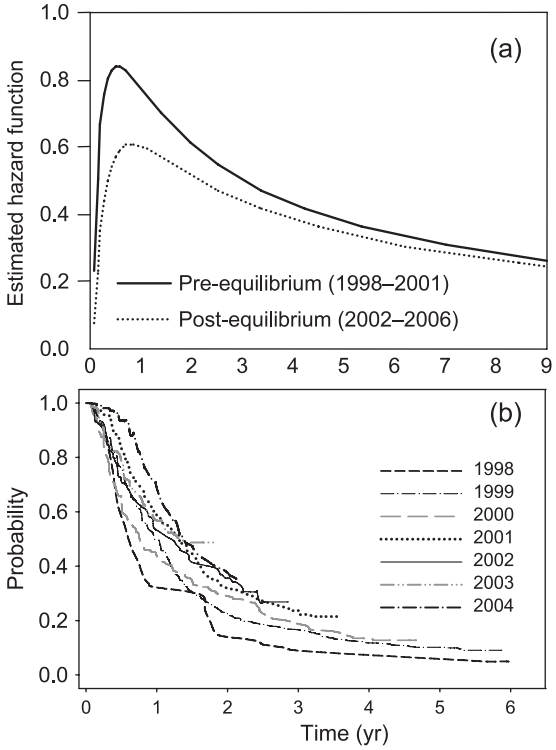

Fig. 1 (a) Estimated log-normal hazard function for all fine roots at the Duke FACE (free air CO_2 enrichment) site located in Durham, NC, USA. This hazard function illustrates that fine roots initiated during the first 3 yr of this minirhizotron experiment (i.e. pre-equilibrium) had a greater hazard of dying compared with roots that were initiated during the last 5 yr (i.e. post-equilibrium). The right-skewed nature of the hazard distribution suggests the presence of both long- and short-lived root pools. (b) Kaplan–Meier survivorship curves of fine roots initiated during each of the first 6 yr of the minirhizotron experiment. Median longevity of fine roots increased with increasing duration of the study.

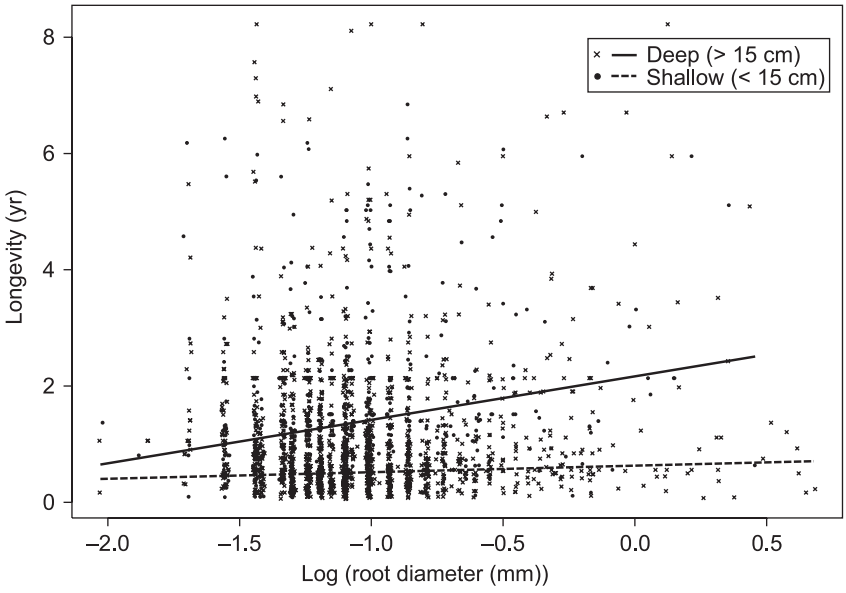

Fig. 2 Relationship between root longevity (yr) and root diameter for roots produced in shallow (0–15 cm) and deep soil (15–30) at the Duke FACE site from Fall 1998 through January 2007. Longevity was determined based on visual observations of roots using minirhizotrons. Root longevity was not influenced by diameter in shallow soil, but increased with diameter in deeper soil. These data point out the heterogeneity in longevity of fine roots (< 2.0 mm in diameter).

that this effect is likely to be small. Results of isotopic and other core-based approaches are only accurate if all of the roots in a soil core are recovered and contribute to a sample mean; difficulties in recovering the finest, most ephemeral roots from soil cores are well known but not well quantified (Pierret *et al.*, 2005; Metcalfe *et al.*, 2007). There may also be sampling errors inherent to the minirhizotron technique that are not addressed in the current modeling study. Our data from Duke FACE indicate that the first roots to colonize the minirhizotron tube surface are the most dynamic and that it may take several years for the less dynamic, longer-lived roots to reach the surface and therefore for the population of roots sampled to reflect bulk soil (Fig. 1). This problem, although not explicitly addressed in the study by Guo *et al.*, likely contributes to the short turnover times reported in many minirhizotron studies of short duration (i.e. ≤ 2 yr).

Future directions

Guo *et al.* have illuminated some of the important drawbacks inherent to minirhizotron and isotopic techniques. It now seems clear that direct application of (mass-based) isotopic results to estimate fine root longevity is unwise. On the other hand, it is equally unwise to apply median root longevity values derived from short-duration minirhizotron studies to quantify flux of C and N through forest soils. We suggest that survival analysis conducted on units of root volume, instead of the individual roots themselves, could be used to better quantify turnover of fine root mass in minirhizotron studies. In longer-duration minirhizotron studies, a larger proportion of fine roots can be observed until death and therefore it is possible to estimate more accurately mean, rather than median, longevity.

The current study does not attempt to explain why fine root longevity estimates derived from sequential soil coring methods are also much lower than longevity estimates determined isotopically. Because both approaches are based on roots obtained with soil cores and since they both measure residence time of either carbon or biomass, they should be subject to similar sampling biases and should yield similar results. A review by Gill & Jackson (2000), based mainly on soil coring and ecosystem budgeting techniques, indicated that average longevity of tree fine roots is approx. 1.8 yr, far shorter than isotopic estimates. Indeed, a compartment flow study conducted at the Duke FACE experiment suggested that fine roots lived approx. 3 yr (Matamala & Schlesinger, 2000), whereas results from an isotopic study in the same plots reported root longevities of 4.2–5.7 yr (Matamala *et al.*, 2003).

The possibility that C spends a significant amount of time in storage pools, or that significant quantities of carbon-containing compounds are transported from senescing to nascent roots, needs to be further explored (Luo, 2003). Mobilization of old carbon to construct new roots could contribute to overestimation of fine root longevity estimates derived from isotopic studies (Luo *et al.*, 2004).

In the end, understanding the biology of the plant/soil continuum will require a better grasp on controls and sources of the considerable heterogeneity that characterizes the fine root pool (Fig. 2; Hishi, 2007). As Guo *et al.* have shown, branch order may account for a large proportion of variation in fine root longevity. Heterogeneity from root herbivory might also be important, considering that the smallest, most ephemeral roots are also the most nutritious (Pregitzer *et al.*, 2002). Proximity to carbohydrate sources, soil moisture, temperature, chemistry, microbiological activity, and depth are all likely to be important too. One can easily imagine the existence of fine root populations, even within a common branch order, that are adapted to function best within a particular location or at a particular time relative to tree ontogeny or the passing of the seasons. Some might argue that this heterogeneity is not important for ecosystem carbon and nitrogen budgeting exercises. But considering the physiological linkages and feedbacks between forest carbon and nitrogen dynamics and resource uptake through the finest, most ephemeral roots along with their fungal symbionts, such a view may be unwise.

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Key words: coring, fine root, isotope, longevity, minirhizotron, root turnover.

Letters

The endodermis: a horsetail's tale

Cui *et al.* (2007) provided a compelling explanation of the mechanism controlling endodermis specification in the root of *Arabidopsis*. The mechanism consists of a positive feedback loop that involves the transcription factors SHORTROOT (SHR) and SCARECROW (SCR). According to Cui *et al.*'s (2007) model, endodermal specification is achieved by export of SHR from the root stele into endodermis precursors that express *SCR*. There, SHR binds to SCR and together they promote production of more SCR, which traps all SHR exported from the stele so that none can escape beyond the endodermal layer to trigger the formation of additional endodermises. Interestingly, Cui *et al.* (2007) also identified potential functional homologs of *SHR* and *SCR* in the rice root. Based on these findings, they suggested that an SHR-SCR feedback loop-type mechanism of endodermal specification might have been acquired early in land plant evolution and could be conserved across most vascular plants. If this were true, then Cui *et al.*'s (2007) model could apply to the development of the endodermis in most, if not all, tracheophytes. The identification, in *Pinus sylvestris* roots, of an SCR putative homolog expressed in the quiescent center equivalent (stele and root cap initials) and in the endodermis (Laajanen *et al.*, 2007) suggests that SCR has comparable functions (maintenance of root radial patterning and endodermal specification) in gymnosperms and angiosperms. This indicates that Cui *et al.*'s (2007) model could indeed apply at least to all seed plants, and provides additional impetus for the hypothesis of a conserved mechanism controlling endodermal specification across all vascular plants.

Discussing this exciting possibility, Dolan (2007) pointed out that there are some rare exceptions from the simple structure

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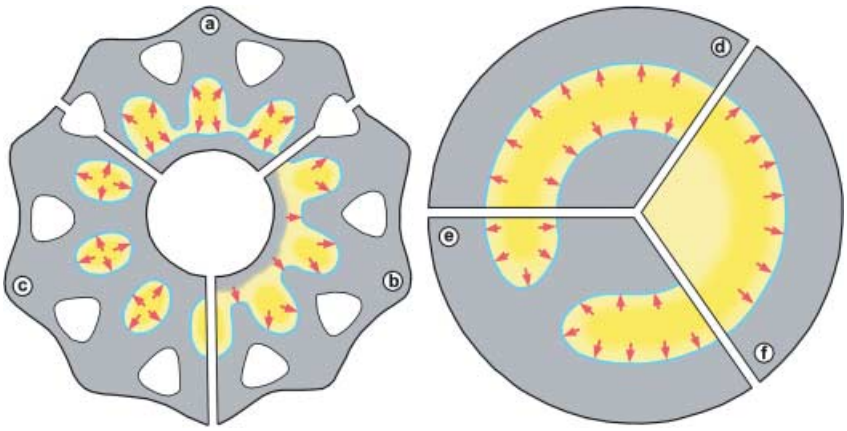

Fig. 1 Application of an SHR-SCR positive feedback loop-type mechanism of endodermis specification, hypothetically conserved across vascular plants, to explain the variety of endodermal structures of vascular plants. Yellow, vascular tissue, procambium, nonconducting tissue of vascular/procambial specification or general site of *SHR* homolog expression. Blue, endodermis or site of *SCR* homolog expression. Red arrows, direction of export of SHR homolog. (a) *Equisetum* stem internode, bi-endodermal; (b) *Equisetum* stem internode, outer endodermis only; (c) *Equisetum* stem internode, individual endodermal layers for each vascular bundle; (d) *Equisetum* stem node, bi-endodermal; (e) siphonostelic fern stem, bi-endodermal, with leaf gap; (f) monocot root and stem of fern with outer endodermis only.

with one endodermal layer seen in the *Arabidopsis* root. He cited the stems of some *Equisetum* (horsetail) species that develop two endodermal layers, suggesting that 'these horsetails have apparently tinkered with their *SCR* and *SHR* genes', and intimating that some variation in Cui *et al.*'s (2007) model based on *Arabidopsis* was needed to explain endodermal specification in *Equisetum*. A close look at the anatomy of *Equisetum* demonstrates that if the mechanism uncovered by Cui *et al.* (2007) is indeed conserved across vascular plants, then the double endodermis of *Equisetum* does not necessitate significant departures from that model, providing additional support for it. Discussion of this alternative opens new perspectives, outlined later in this paper, for understanding other types of endodermal structure encountered frequently across the spectrum of plant diversity and which break away from the anatomical norm established by the roots of dicotyledonous angiosperms.

Two endodermal layers

In Cui *et al.*'s (2007) model, endodermal specification is achieved by active export of SHR from the stele located at the center of the root, centrifugally into endodermis-cortex precursors that express *SCR*. In bi-endodermal stems of *Equisetum*, the two endodermal layers (outer and inner endodermis) develop on either side of a ring of vascular tissue, whether the latter is dissected into discrete bundles (in internodes; Fig. 1a) or not (at nodes; Fig. 1d) (Ogura, 1972). If the same source-sink pathway of SHR transport demonstrated in *Arabidopsis* functions in horsetails, then given the position of vascular tissues between the two endodermal layers, no alteration is necessary for Cui *et al.*'s (2007) model to explain this structure. In these bi-endodermal stems, the SHR homolog would simply have to be exported away from the vascular tissues, just as in Cui *et al.*'s (2007) model. And since, in these *Equisetum* stems, the vascular tissues do not occupy the center of the stem, export of SHR homolog away from the vascular tissues would be bidirectional – centrifugal and centripetal (Fig. 1a,d). If the *SCR* homolog is expressed on both sides of the ring of vascular tissue simultaneously, then together the two processes would result in specification of two endodermal layers.

The bi-endodermal structure of *Equisetum* is far from being a rare exception. Similar structures characterize the stems of most ferns with a siphonostelic architecture (as defined by Beck *et al.*, 1982; see also Bower, 1935; Ogura, 1972). If an SHR-SCR feedback loop-type mechanism is plesiomorphic and conserved across all vascular plants, then the development of an outer and inner endodermis could be explained in all these plants by the same processes as described earlier: export of SHR homologs from, and expression of *SCR* homologs around, the hollow cylinder of vascular tissue, both centrifugally and centripetally (Fig. 1e). Indeed, there is no reason why SHR would not be exported away from the vascular tissue in all directions; and in fact, this is what happens in the *Arabidopsis* root, where SHR is exported into neighboring tissue all the way around the vascular tissue.

Discrete vascular strands with individual endodermal layers

The same model (export of SHR away from vascular tissue in all directions) could also explain the anatomy of *Equisetum* stems that exhibit an individual endodermal layer around each vascular bundle (Fig. 1c). Given the organization of the vascular tissue of *Equisetum* internodes into discrete strands, this endodermal arrangement makes even more sense in light

of Cui *et al.*'s (2007) model, requiring the same type of centrifugal export of SHR homolog around each discrete vascular strand and expression of *SCR* homolog around the latter, as seen in the single, centrally located vascular strand of the *Arabidopsis* root. In fact, this second type of endodermal structure encountered in *Equisetum* raises questions about the mechanisms controlling the radial patterning of tissues in those specimens (discussed earlier) that have a bi-endodermal structure (Fig. 1a). What is the identity of cells in the narrow strips encompassed by the two endodermal layers between neighboring vascular bundles? These cells must either produce SHR homolog themselves, or they must relay tangentially the SHR exported from vascular bundles. In either case they must then be exporting SHR centrifugally and centripetally to ensure the continuity of the two endodermal layers between neighboring vascular bundles. If these cells produce SHR homolog, then that would make them similar to vascular tissue. If they just relay SHR homolog, then they certainly do not express the *SCR* homolog, otherwise they would differentiate as endodermal cells themselves.

Back to one endodermal layer

The most puzzling endodermal feature of *Equisetum*, as seen through the perspective of an SHR-SCR positive feedback loop model, is, in fact, illustrated by those specimens that develop only one continuous endodermal layer, the outer endodermis (Fig. 1b). Given that these specimens have the same vascular architecture consisting of discrete bundles, and assuming that SHR/*SCR* homologs are generally exported/expressed in all directions around vascular tissue, the development of an outer endodermis alone implies alterations to the basic model. This could mean suppression of SHR export toward the nonvascular center of the stem or lack of SCR synthesis to the inside of the ring of bundles, or both. Similar situations, involving a hollow cylinder of vascular tissue (with or without leaf gaps) with what looks like ground tissue at the center and provided only with an outer endodermis (Fig. 1f), have been documented in several ferns in the Ophioglossaceae, Osmundaceae, and Vittariaceae (Ogura, 1972).

A possible explanation for the structure of all these pteridophyte stems that have only an outer endodermis, despite the fact that their vascular tissues do not go all the way to the center, could come from monocot roots. Some monocot roots are described as having a central pith, but a quick look at their development shows that the parenchymatous tissue that forms a central column is derived from procambium (Esau, 1977). Therefore, these apparently siphonostelic roots actually have cryptic protosteles, and, although it consists of parenchyma, their 'pith' has vascular tissue identity. As such, the 'pith' expresses genes characteristic of vascular tissue (or procambial) identity, such as an *SHR* homolog, and does not express the *SCR* homolog which is expressed only outside the ring of vascular tissue (Fig. 1f), as shown by Cui *et al.* (2007) for the rice root. Could it be that something similar happens in fern and horsetail stems with only an outer endodermis? Perhaps the proteome of cells in the nonvascular center of those stems somehow specifies them more as procambial cells than anything else. This explanation cannot be ruled out entirely before it is thoroughly tested – so there is much more to do in the study of molecular controls on development in seed-free plants, a rarely approached direction of plant biology.

A universal motif for specification of boundary layers

Accumulating evidence suggests that the SHR-SCR feedback loop mechanism could represent a universal motif responsible for the specification of a boundary layer around vascular tissues throughout the plant body, whether that layer is a functional endodermis (i.e. with suberized Casparian strips, as seen in roots and some stems), a more or less well defined starch sheath (in other stems; Esau, 1977), or a vascular bundle sheath (in leaves). Such evidence comes from studies in *Arabidopsis*, which have shown that SHR and SCR are required for the formation of a normal boundary layer in stems (Fukaki *et al.*, 1998), and that SCR is expressed in the layer immediately adjacent to the vascular tissues in stems, as well as in the bundle sheath cells of all veins (Wysocka-Diller *et al.*, 2000). If real, the universality of the SHR-SCR feedback loop mechanism in specifying boundary layers throughout the plant body and in all plants raises two important issues. One of these issues is that if the mechanism is common to all boundary layers, then specification of a functional endodermis would require the participation of additional mechanisms downstream of the SHR-SCR feedback loop.

The other issue has to do with the position of the boundary layer in dicot stems where this layer goes around the stele only to the outside of the vascular bundles. This is not what we would expect to see based on the distribution of boundary layers in nonprotostelic fern stems (as discussed earlier) and *Equisetum*. Seed plant stems have eusteles characterized by a ring of discrete vascular bundles arranged around a central pith. If specification of the boundary layer in these stems is controlled by the same SHR-SCR feedback loop mechanism, then we would expect to find this boundary layer forming individual sleeves either around each vascular bundle of the eustele (as in the *Equisetum* stems with individual endodermises around the vascular bundles), or on both sides of the ring of vascular bundles (as in bi-endodermal *Equisetum* and fern stems). Instead, dicot stems are more like those *Equisetum* stems provided with only an outer endodermis, and like the monocot roots with 'pith'. However, unlike the latter, the pith of dicot stems does not have vascular tissue identity so it would be interesting to know whether it expresses *SHR* even in the absence of vascular tissue identity. If so, that would represent additional evidence for the universality of the SHR-SCR feedback loop mechanism, but it would also intimate a certain

degree of uncoupling between *SHR* expression and vascular tissue identity.

Exaptation?

Going back to the initial hypothesis of a putative mechanism of endodermal specification conserved across all vascular plants, we can ask the following: what were the molecular origins in the evolution of such a mechanism? *SHR* and *SCR* homologs have been characterized in the gametophyte phase of the moss, *Physcomitrella patens* (Kitagawa *et al.*, 2006, 2007). However, moss gametophytes lack an endodermis and the functions of the two genes in *Physcomitrella* are unknown at present. Is it possible that *SHR* and *SCR*, conserved across embryophytes, illustrate the exaptation (a more elegant term for recruitment) of genes to new functions, during plant evolution, between the gametophyte and sporophyte phases, in the transition from the bryophytic condition to the polysporangiophyte-tracheophyte body plan? Shubin & Marshall (2000) have advocated a view of different organisms as mosaics of similar genes redeployed in different functional contexts during the evolution of gene regulatory interactions. Indeed, exaptation of function of existing genes (rather than evolution of new genes) at the transition between gametophyte-dominated and sporophyte-dominated life cycles, in embryophytes, is gaining increasing support from comparative genomic studies, which suggest that most of the gene families involved in developmental patterning in angiosperms are also found in mosses (Bowman *et al.*, 2007). An instance of exaptation of a molecular pathway from mosses to vascular plants is illustrated by the homologous *PpRSL1* and *AtRHD6* genes, which control, respectively, development of gametophyte rhizoids in *Physcomitrella* and root hair development in the *Arabidopsis* sporophyte (Menand *et al.*, 2007). As pointed out by Menand *et al.* (2007), such evolutionary transitions could be at the origin of the morphological radiation of the sporophyte phase early in the evolutionary history of vascular plants.

And back to *Equisetum*

Finally, not only is the type of endodermal structure a species-diagnostic character in *Equisetum* (Ogura, 1972, provides the most comprehensive, although not exhaustive, summary of the taxonomic distribution of the three endodermal structure types), but species of this genus present us with the very few documented examples of transition from one type of endodermal structure to another along the same plant. As documented by Bierhorst (1971) and discussed by Ogura (1972), the underground rhizomes and the bases of aerial stems of 12 *Equisetum* species (among them *E. hyemale*, *E. kansanum*, *E. ramosissimum*, *E. myriochaetum*, *E. laevigatum*, *E. robustum*) feature individual endodermal layers around each bundle, whereas, distally in the same plants, the aerial stems have an inner and an outer endodermis. Similarly, whereas the rhizomes and bases of aerial stems of *E. sylvaticum* exhibit an inner and an outer endodermis, aerial stems are characterized distally by a single outer endodermis. Interestingly, in all documented cases, the basal, underground part of the vertical stem has the same endodermal structure as the horizontal rhizome, and the transition to the endodermal structure of the aerial portion occurs at or near ground level (Bierhorst, 1971). The general pattern of these transitions between endodermal structure types in *Equisetum* seems to reflect a relaxation of the endodermal control on apoplastic transport from the below-ground to the above-ground parts of the plants. These structural transitions indicate that, whether it is a conserved SHR-SCR feedback loop mechanism or not, the mechanism that regulates endodermal specification in *Equisetum* is under environmental control. Thus, if SHR/SCR homologs were identified in *Equisetum*, this genus could provide a useful model system for understanding environmental controls on gene expression.

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Key words: *Arabidopsis*, endodermis, *Equisetum*, exaptation, ferns, monocots, root, SCARECROW, SHORTROOT.

Meetings

Automated soil respiration measurements: new information, opportunities and challenges

Automated Soil Respiration Workshop – a Terrestrial Ecosystem Response to Atmospheric and Climate Change (TERACC) sponsored workshop, Durham, New Hampshire, USA, September 2007

Soils are the largest carbon pool in terrestrial ecosystems, and soil respiration is the major pathway of carbon transfer from soil to the atmosphere. Measuring and predicting soil respiration has been challenging because the CO_2 efflux from soil integrates several complex below-ground processes (Zhou & Luo, 2006). Current models of soil respiration lack a theoretical underpinning with which to predict how fluxes reflect different plant and microbial CO_2 sources and mechanisms of CO_2 production. Recent technological advances in automated soil respiration (ASR) systems are generating unprecedented numbers of high temporal-resolution observations (Savage & Davidson, 2003). Automated soil respiration provides valuable information that is often missed with less frequent manual measurements, and presents the opportunity to move beyond empirical (gap-filling) models towards a predictive understanding of the key mechanisms that determine soil respiration fluxes. However, these continuous measurements present new challenges in that they require the additional management of complex equipment and large data sets as well as novel analytical approaches.

In September 2007, researchers met in Durham, New Hampshire, USA, for a workshop on ASR measurements (http://www.umaine.edu/teracc). The overall goal of the workshop was to initiate communication within the ASR measurement community and to provide a foundation for future research and syntheses studies. The meeting focused on: how automated measurements are advancing our understanding of soil respiration processes; challenges for the quality analysis (QA) and quality control (QC) of large data sets; and identifying current knowledge gaps and future research direction on soil respiration. The following questions provided the structure for the workshop.

- What are we learning from automated measurements of soil respiration?
- How do we know when we are making good measurements: QA and QC of large data sets?
- What are the best ways to analyze and model ASR data?
- What are the future research directions for automated chamber measurement studies and syntheses?

'... fast-changing diel patterns and dynamic responses to environmental events like precipitation pulses require ASR to quantify short-term responses.'

New insights from automated soil respiration measurements

Automated soil respiration techniques provide reliable and continuous measurements that can be deployed in complex terrain, near eddy covariance towers and at manipulation experiments. Several research groups and networks have begun ASR measurements that are starting to provide a solid basis for site-to-site intercomparisons (e.g. Ameriflux, Asiaflux, Carboeurope), and these efforts will be crucial for interannual comparisons across sites in the years to come.

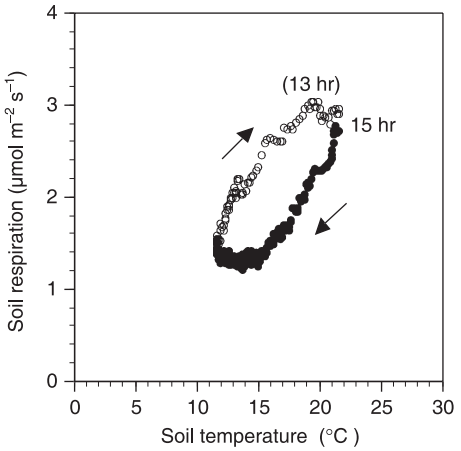

Fig. 1 Example of a diel pattern of soil respiration and soil temperature using automated soil respiration (ASR) measurements in a mixed pine-oak forest at the James Reserve, CA, USA. The figure shows a lag of 2 h between maximum soil respiration (13 h) and maximum soil temperature (15 h). Open circles represent increasing temperatures and black circles represent decreasing temperatures. Arrows indicate the clockwise direction of a hysteresis loop. Data were derived from soil CO_2 profile measurements at 5-min intervals (R. Vargas & M. F. Allen, unpublished).

Automated soil respiration measurements provide information about temporal complexity at multiple scales where changes in biophysical factors play a major role (Baldocchi *et al.*, 2006). The re-occurring new insights from ASR data highlight the importance of diel patterns and time lags, and the quantitative evaluation of seasonal dynamics. Several talks included discussions of diel patterns of soil respiration and temperatures with lags up to 5 h and showing hysteresis effects (Fig. 1). The lags were related to changes in plant phenology and photosynthesis, but the presence of snow, changes in turbulence conditions near the soil surface or differential diffusivity of soil CO_2 could also influence these patterns. Recent studies, including isotope studies, natural spatial gradients and manipulations by girdling or controlling temperature, presented evidence of the role of photosynthesis in the diurnal patterns of soil respiration.

Participants in the workshop recognized that the same processes that create day/night differences in soil respiration fluxes might not be the ones controlling seasonal or interannual variability. In addition, fast-changing diel patterns and dynamic responses to environmental events, such as precipitation pulses, require ASR to quantify short-term responses. Long-term ASR measurements are providing observations of soil respiration under a wide range of meteorological and phenological conditions to address these questions. With this information, researchers are selecting appropriate time intervals for exploring the role of driving variables (e.g. temperature), when others (e.g. phenology, moisture) are not changing, to understand mechanisms of soil respiration under different environmental conditions.

Quality analysis and quality control

There is an increasing interest in clearly defined and common QA/QC procedures for large data sets accumulated from ASR measurements. Relatively simple procedures, such as checking sensor values and flow meters, can be implemented to screen data with a view to eliminate obviously bad data. Immediate processing of the data, including rapid visualization of flux tracings using appropriate software, can help to organize and detect errors of equipment failure in order to minimize data gaps. Wireless connectivity could further help with screening data in near real-time. Corrections to absolute flux rates may be improved by the additional frequent measurements of chamber volume and water vapor concentrations in the field. A combination of both visual and quantitative screening of data is recommended as needed. Automated data cleaning and gap-filling analysis for ASR measurements are an opportunity to develop algorithms that may analyze historical data for data QA/QC. A follow-on effort was organized to define standard QA/QC protocols, including a common list of meteorological variables, especially to support comparisons among sites and when different techniques or chamber designs are used to calculate soil respiration rates.

Analyzing and modeling soil respiration

Automated soil respiration chambers can be placed at multiple points in space to generate a continuous time series of soil respiration (Drewitt *et al.*, 2002), and profile measurements of CO_2 concentrations provide vertical information of production and transport of CO_2 at different soil depths (Tang *et al.*, 2003; Davidson *et al.*, 2006). The large data sets resulting from these measurements require rigorous techniques for data assimilation to synthesize information and construct robust mechanistic models. As a result, the parameters of the models can be constrained by high-quality data collected at a time-step close to specific needs.

A primary challenge in understanding the controls on soil respiration is to separate the measured CO_2 flux into its autotrophic and heterotrophic components, and then to quantify how each source responds to changes in the environment (e.g. light, temperature and moisture), substrate supply and quality, and plant phenology. The covariance of these driving variables often limits the ability to predict parameters driving soil respiration. Automated soil respiration measurements, in combination with isotopic techniques such as labeling (Carbone & Trumbore, 2007), background abundance (with ^{14}C) and continuous ^{13}C measurements offered with tunable diode lasers, can separate autotrophic and heterotrophic source contributions. Additionally, ASR measurements can be combined with studies across gradients and appropriate experimental manipulations (e.g. cold-block phloem, girdling, root trenching) to assess independently the controls on these sources.

Future directions

Currently, advances in the development of mechanistic models for soil respiration are hindered by both a lack of observations and the need to design experiments to emphasize the role of a single driving variable. Special challenges include developing ways to understand, in greater detail, the role of substrate supply for both autotrophic and heterotrophic respiration sources (Barbour *et al.*, 2005). Again, discussions highlighted applying a combination of approaches, including the simultaneous deployment of CO_2 concentration profile sensors and chamber measurements, isotopic applications, manipulations, and high-frequency measurements of root and mycorrhizal dynamics to test hypotheses about drivers of soil respiration variability. Manipulations should be expanded to include global change experiments such as warming and altered nutrient and water regimes (Pendall *et al.*, 2004). Such manipulation experiments have been applied in the past and are ongoing, but the introduction of ASR measurements affords an opportunity to investigate how these manipulations affect autotrophic and heterotrophic processes at timescales from hours to seasons. An example of this is the incorporation of ASR measurements in free air CO_2-enrichment experiments (Liu *et al.*, 2006). Intensive measurements of soil respiration with observations of soil temperature, soil water status, soil carbon pools, root mass data and plant-allocation patterns at key sites are important for comparison of sites with different vegetation and climate.

There are emerging opportunities for cross-site and regional comparisons or synthesis analysis of ASR measurements. These comparisons range from research in QA/QC analysis and chamber designs to investigations of seasonal and diel patterns in soil respiration. This is an exciting time for studying soil respiration processes and model development, as demonstrated by the new information presented at this workshop.

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Key words: automated soil respiration (ASR), automated measurements, autotrophic, heterotrophic, soil respiration, Terrestrial Ecosystem Response to Atmospheric and Climatic Change (TERACC).

Rhizosphere studies from the nanoscale to the globe

Rhizosphere 2, Montpellier, France, August 2007

After the successful Rhizosphere 2004 Conference in Munich, Germany (Sen, 2005) it was evident that there is a niche for regular meetings devoted to this multidisciplinary area. In August, over 500 researchers met in Montpellier, France for Rhizosphere 2 (http://www.montpellier.inra.fr/rhizosphere-2/). The conference attracted a mixed audience of botanists, microbial ecologists, and soil scientists who each have their

own focal points and dynamics. This can result in a centrifugal tendency in rhizosphere sciences, where the central questions and the newest research tools of the underlying disciplines, more than the rhizosphere itself, are driving the research approaches. There were some examples of this when results of highly sophisticated molecular ecology studies of soil micro-organisms were presented without much attention being paid to the plant root and soil. This clearly illustrates the importance and difficulties of conciliating the integrity and multidisciplinary character of rhizosphere sciences. Nevertheless, I enjoyed a rich diversity of reports in Montpellier, a selection of which are highlighted below.

'... Al tolerance is regulated by LMWOA transport into the rhizosphere – which brings the rhizosphere and the micro-organisms that inhabit the rhizosphere into prominence again'

Novel methods and mathematical models

A research field whose object of study is still, after more than a century, loosely defined as 'the soil immediately adjacent to plant roots as influenced by the plant' is very eager for new methodological developments. Here I should first mention the handbook of methods used in rhizosphere research (Luster & Finlay, 2006; http://www.rhizo.at/default.asp?id=574&lid=2). Several sessions of the conference were devoted to novel methods. Anke Herrmann (Newcastle University, UK) introduced NanoSIMS, a method that links very high-resolution microscopy (spatial resolution around 50 nm) with isotope analysis (up to five ion species can be detected simultaneously). She described the advantages of this method, which were further demonstrated in a presentation by Elisabeth Stockdale (Newcastle University, UK) who has used the method to investigate the competition for amino acids between microbiota and plant roots in the rhizosphere. Herrmann also highlighted the limitations of NanoSIMS, such as the creation of potential artefacts during sample preparation and the limited two-dimensional area that can be analysed. The importance of a three-dimensional view of the rhizosphere was particularly stressed by Iain Young (University of Abertay, Dundee, UK). He beautifully illustrated and discussed the issue of scaling and the problems that arise during scaling if the soil matrix is treated as homogeneous using averaging techniques. Rapid technical progress of various omics approaches was also covered with presentations on pyrosequencing-based 454 sequencing (Janet Jansson, Swedish University of Agricultural Sciences, Uppsala, Sweden), phylochips (George Kowalchuk, NIOO-KNAW, Heteren, the Netherlands) and metatranscriptomics (Roland Marmeisse, University of Lyon, France). In parallel with all this technical progress, mathematical models could also make a contribution by bringing more rigour to the field. Andrea Schnepf (University of Natural Resources and Applied Life Sciences, Vienna, Austria) presented a mycorrhiza uptake model (Schnepf & Roose, 2006) where hyphal architecture (hyphal branching patterns are different in different genera of arbuscular mycorrhizal fungi) and the characteristics of the fungal uptake system turned out to be important parameters, demonstrating how models can provide guidance to the experimentalists. Another model was presented by Wilfred Otten (University of Abertay, Dundee, UK) who highlighted the importance of root architecture (spatial and temporal dynamics of root systems) for pathogen control in the rhizosphere. Otten showed how changes in root architecture can be linked to switches in pathogen behaviour from noninvasive to invasive. Such models could then be used to evaluate control strategies of soil-borne plant diseases.

Expensive exudates

The soil remained a recalcitrant matrix for several studies. It has been known for a long time that low-molecular-weight organic acids or anions (LMWOAs), mainly malate and citrate, were exuded at the root tip in response to aluminium (Al) toxicity. However, there has always remained the nagging uncertainty of whether the mechanism could work as suggested, because of discrepancies between LMWOA concentrations at the root apex and concentrations that are necessary to explain the tolerance of certain genotypes (Parker & Pedler, 1998). Because of such problems of quantification, as a consequence of sorption on soil particles and breakdown in the rhizosphere, the field has become more molecular. Leon Kochian (Cornell University, Ithaca, USA) provided an overview of the developments in the field, describing a new tolerance gene in sorghum (*Sorghum bicolor*) affecting citrate efflux. His work showed that different cereals (C_3 barley (*Hordeum vulgare*) and C_4 sorghum) produce different LMWOAs (malate and citrate, respectively). However, with all the clarifications that molecular biology provided, it is still clear that Al tolerance is regulated by LMWOA transport into the rhizosphere – which brings the rhizosphere and the micro-organisms that inhabit the rhizosphere into prominence again. LMWOAs are not only important in conferring tolerance to Al but also play a role in regulating phosphorus (P) dynamics in the rhizosphere. The ability of plants to produce LMWOAs as a way to cope with P deficiency was emphasized by Hans Lambers (University of Western Australia, Crawley, Australia). Soils in southwest Australia are extremely P-deficient, up to the point that neither a very high root hair density nor mycorrhizal associations are sufficiently effective to provide plants with adequate P. Lambers referred to a study by Parfitt (1979), who had shown that

adsorption of small amounts of phosphate on iron oxides can be too strong to allow uptake by (mycorrhizal) roots and that P desorption through organic anions is much more effective. Cluster roots and dauciform roots (Shane *et al.*, 2006), which have both evolved repeatedly, therefore constitute the better solution on iron (Fe)-rich, P-poor soils. The spatially and temporally localized exudation of citrate and malate (these plants avoid acidifying the rhizosphere, which could negate the effects of the anions, and release potassium (K^+) as a counter-ion, as K^+ is hardly limiting in these soils) allows the plant to obtain its P. However, the strategy is very expensive and carbon (C) costs are about twice as high as those in a mycorrhizal strategy. Lambers drew two relevant implications from his studies. First, the acquisition of P must be a continuous process, so there is little chance of down-regulation of P uptake and consequently of down-regulation of citrate and malate exudation. The price that such plants pay is that they develop rapid P toxicity symptoms in environments where other plants would grow without difficulty (Shane *et al.*, 2004). Understanding strategies for both efficient P acquisition and avoidance of P toxicity could help in developing new cropping systems and new crops, which is imperative considering the low use efficiency of applied P fertilizer, especially in Fe-rich soils, and the limited global stocks. Secondly, along the nutrient availability gradient (Read & Perez-Moreno, 2003), P replaces nitrogen (N) as the most limiting element. On sites that have slightly higher P availability, ectomycorrhizal plants (Casuarinaceae) occur that simultaneously produce cluster roots. It is not known for these plants to what extent ectomycorrhizal fungi or cluster roots contribute to P mobilization and whether the two strategies are traded off. I imagine that many attendants felt a strong desire to see (and investigate!) this unique vegetation and the next rhizosphere conference (planned at Perth, in 2011) would provide an ideal opportunity for that.

Arsenic

The main elements in rhizosphere studies discussed during the conference were C (as the motor of the rhizosphere system), N (including N_2 fixation, nitrification and denitrification), P and Al (in relation to both LMWOA and mycorrhizal associations), heavy metals, and arsenic (As). Presentations from Yong-Guan Zhu (Chinese Academy of Sciences, Beijing, China) and Doris Vetterlein (UFZ and Martin Luther University, Halle, Germany) indicated the linkages between the behaviour of As in soils and plants in relation to that of other nutrients. The presence of As in Fe plaques surrounding roots reduces As toxicity to plants, but Fe reduction could subsequently release As. Nitrate reduction simultaneously involves Fe oxidation, which simultaneously diminishes As toxicity and potentially increases N_2O emission. Vetterlein indicated that not only redox reactions in the soil are important for As speciation; in plants As^{5+} is also rapidly reduced to As^{3+}, complexed with thiols and sequestered in vacuoles or transported to the soil via As^{3+} efflux.

A global perspective

The scaling up of rhizosphere processes to global scales was introduced by Bruce Hungate (Northern Arizona University, Flagstaff, USA). He presented several studies where rhizosphere processes constrained ecosystem responses to various drivers of global change, noting that both the spatial dimension and the temporal dimension were important. Bridging these scales and understanding the feedbacks between plant and soil responses as mediated through rhizosphere processes are essential to improve our understanding and prediction of global environmental change.

With over 100 presentations and around 450 posters, it is impossible to do justice to the wealth of diverse topics presented during the conference. I therefore apologise to those contributors whose interesting studies could not be mentioned. It is also clear from these other contributions that rhizosphere sciences are flourishing.

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