References

Dickie IA. 2007. Host preference, niches and fungal diversity. *New Phytologist* **174**: 230–233.

Fitter AH. 2006. What is the link between carbon and phosphorus fluxes in arbuscular mycorrhizas? A null hypothesis for symbiotic function. *New Phytologist* **172**: 3–6.

Govindarajulu M, Pfeffer PE, Jin H, Abubaker J, Douds DD, Allen JW, Bücking H, Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435**: 819–823.

van der Heijden MGA, Scheublin TR. 2007. Functional traits in mycorrhizal ecology: their use for predicting the impact of arbuscular mycorrhizal fungal communities on plant growth and ecosystem functioning. *New Phytologist* **174**: 244–250.

Koide RT, Courty P-E, Garbaye J. 2007. Research perspectives on functional diversity in ectomycorrhizal fungi. *New Phytologist* **174**: 243–246.

Lilleskov EA, Parrent JL. 2007. Can we develop general predictive models of mycorrhizal fungal community–environment relationships? *New Phytologist* **174**: 250–256.

Martin F, Slater H. 2007. *New Phytologist* – an evolving host for mycorrhizal research. *New Phytologist* **174**: 225–228.

Munkvold L, Kjøller R, Vestberg M, Rosendahl S, Jakobsen I. 2004. High functional diversity within species of arbuscular mycorrhizal fungi. *New Phytologist* **164**: 357–364.

Parrent JL, Morris WF, Vilgalys R. 2006. CO_2-enrichment and nutrient availability alter ectomycorrhizal fungal communities. *Ecology* **87**: 2278–2287.

Rosendahl S, Stukenbrock EH. 2004. Community structure of arbuscular mycorrhizal fungi in undisturbed vegetation revealed by analyses of LSU rDNA sequences. *Molecular Ecology* **13**: 3179–3186.

Rubini A, Riccioni C, Arcioni S, Paolocci F. 2007. Troubles with truffles: unveiling more of their biology. *New Phytologist* **174**: 256–259.

Sanders FE, Tinker PB. 1971. Mechanism of absorption of phosphate from soil by endogone mycorrhizas. *Nature* **233**: 278–279.

Selosse M-A, Duplessis S. 2006. More complexity in the mycorrhizal world. *New Phytologist* **172**: 600–604.

Shachar-Hill Y. 2007. Quantifying flows through metabolic networks and the prospects for fluxomic studies of mycorrhizas. *New Phytologist* **174**: 238–242.

Smith SE, Smith FA, Jakobsen I. 2004. Functional diversity in arbuscular mycorrhizal (AM) symbioses: the contribution of the mycorrhizal P uptake pathway is not correlated with mycorrhizal responses in growth or total P uptake. *New Phytologist* **162**: 511–524.

Wiemken V. 2007. Trehalose synthesis in ectomycorrhizas – a driving force of carbon gain for fungi. *New Phytologist* **174**: 228–230.

Key words: fluxomics, functional traits, fungal communities, fungal diversity, mycorrhiza, predictive models, symbiosis, truffles.

Letters

Quantifying flows through metabolic networks and the prospects for fluxomic studies of mycorrhizas

What is fluxomics?

The goal of fluxomics is to quantify all the metabolic fluxes in a cell, tissue or organism (Sauer *et al.*, 1999; Sauer, 2004). Investigation of the flow of matter through biochemical systems has always been central to the study of metabolism, and analysis of the rates of metabolic transformations – the study of enzyme kinetics – is likewise a long-established aspect of understanding any biological system in detail. However, the conceptual, experimental and computational tools for quantifying the integrated functioning of metabolic networks began to become available only in recent decades, and are still very much under development. Like many other omic approaches, fluxomics has yet to attain the goal of generating comprehensive system-wide data sets. However, progress in the last 10 yr has been rapid and fluxomics has grown beyond its origins in bacterial systems and has begun to make significant contributions to the study of plant systems (Kruger *et al.*, 2003; Schwender *et al.*, 2004a; Ratcliffe & Shachar-Hill, 2006).

How is network flux analysis performed?

The analysis of multiple flows through a network involves both direct and indirect determination of metabolic and transport fluxes. Direct determination of fluxes involves individually measuring the rates of substrate uptake, product secretion, and the accumulation of storage or structural compounds (lipids, carbohydrates and proteins) of known composition. Indirect determination of fluxes is performed in two ways. In the first method, fluxes measured directly are used to deduce other net fluxes; this is done by balancing the influxes and effluxes from individual metabolite pools using the known stoichiometries of biochemical reactions (flux balancing). The second method is based on interpreting the results of labeling experiments. Labeling measurements using radioactive isotopes are made by fractionation or chromatographic separation methods followed by scintillation

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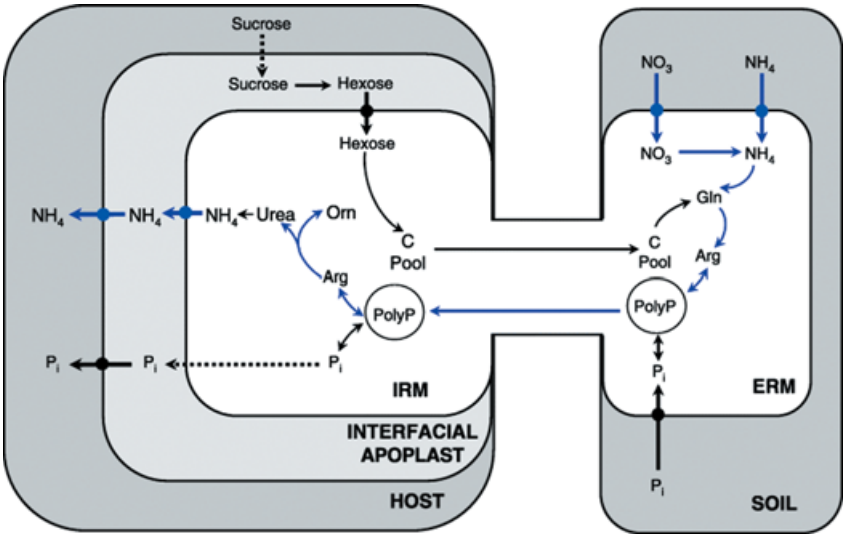

Fig. 1 A model of nitrogen flow in the arbuscular mycorrhizal symbiosis, suitable for initiating a fluxomic analysis. The movements of carbon and phosphorus with which nitrogen (N) fluxes are associated are also outlined. Inorganic N is taken up by the fungal extraradical mycelium and assimilated via nitrate reductase (for nitrate) and the glutamine synthetase/glutamate synthase cycle. It is then incorporated into arginine (Arg), which is translocated along the coenocytic fungal hyphae from the extraradical mycelium (ERM) into the intraradical mycelium (IRM). Arg is then broken down in the IRM, releasing urea and ornithine, which are further broken down by the actions of urease and ornithine aminotransferase. NH_4^+ released from Arg breakdown passes to the host via ammonium transporters or perhaps other mechanisms Chalot *et al*. (2006). P_i, orthophosphate; PolyP, polyphosphate. Reproduced from Jin *et al*. (2005) with the permission of *New Phytologist*©.

counting of different intermediate and product metabolites. Measurements of stable isotopic labeling usually involve ^{13}C (or less commonly ^{15}N or ^{2}H) and are made by nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry. The interpretation of labeling and flux measurement data in terms of multiple fluxes is nontrivial and almost always involves computer-aided modeling. Models are used to estimate fluxes by finding the values that result in a best fit of computed (simulated) to experimental results.

This reliance on fitting to a metabolic model means that a fluxomic investigation requires some prior knowledge of and assumptions about the metabolic architecture of the system. This knowledge is certainly much less complete in mycorrhizas than in many bacteria or model fungi and plants. However, progress in delineating metabolic and transport networks in mycorrhizal systems has been steady in recent years and has reached a point where models can be constructed for the quantitative interpretation of labeling data in the best-studied cases (Chalot & Brun, 1998; Bago *et al*., 2000; Bucking & Shachar-Hill, 2005; Govindarajulu *et al*., 2005). The outline of a working model of central metabolism and transport in the arbuscular mycorrhizal symbiosis that could serve as a starting point for flux analysis is shown in Fig. 1. The advent of mycorrhizal plant and fungal genome sequences will be of enormous help in building and in filling in the molecular mechanisms of such models. When transcript and proteomic data sets for mycorrhizas become much more complete than they are at present – which can be expected to happen sooner rather than later – they too will be important in defining and validating model networks for use in flux analysis.

There are two general approaches to the analysis of metabolic fluxes through a network; they differ in the conditions and the systems to which they are suited as well as in the measurements required and the information they yield. The first approach, dynamic or kinetic analysis, yields metabolic fluxes from the analysis of time-course data on the distribution of isotopic label through the network (Morgan & Rhodes, 2002). The parameters involved in analyzing dynamic labeling experiments include kinetic rate constants (either K_m and V_{max} values or pseudo first-order rate constants) and the concentrations of products and precursors for the enzymatic reactions and transport processes being studied. Some of the pool sizes are typically measured as part of the investigation, and enzyme activities and kinetic properties may be measured or estimated from knowledge about other systems. Dynamic labeling analysis yields estimates of the unknown or uncertain parameters (forward and reverse fluxes, pools sizes, and rate constants) by fitting a kinetic metabolic model to the experimental data. Kinetic models consist of a set of rate equations that describe the fluxes through each metabolic and transport step in the network of interest in terms of the relevant pool sizes and kinetic parameters of enzymes and transporters. The values of these parameters are iteratively adjusted until the model produces simulated time-courses of labeling and pool sizes that best match the experimental data.

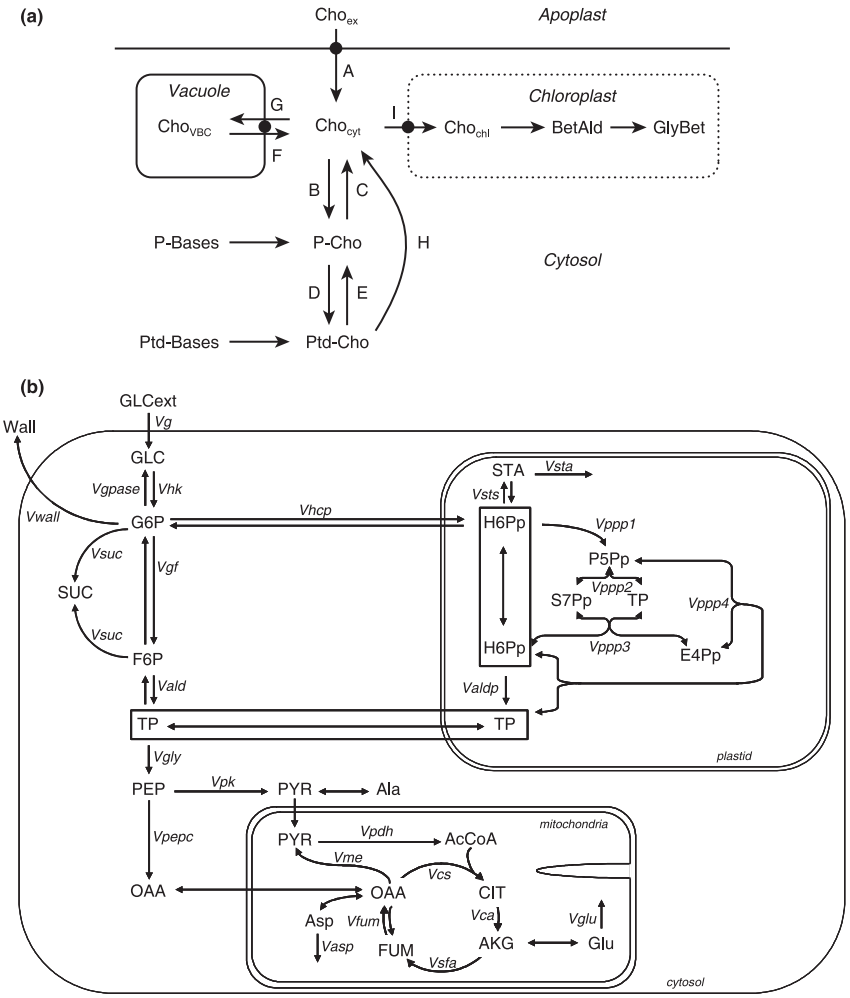

Fig. 2 (a) A model used in a dynamic labeling analysis of the kinetics of glycine betaine synthesis in transgenic tobacco (*Nicotiana tabacum*). Leaf discs were incubated with [^{14}C]choline, and pool sizes as well as labeling time-courses were measured for total choline (Cho; external, Cho_{ex}; in the cytosol, Cho_{cyt}; in the chloroplast, Cho_{chl}; in the vacuole, Cho_{vac}), phosphocholine (P-Cho), phosphatidylcholine (Ptd-Cho), and glycine betaine (GlyBet). V_{max} and K_m values for fluxes B, C, D, E, G and H, and the first-order rate constants for fluxes A, F and I, were obtained by optimizing the fit between the simulated kinetics and the labeling time-courses. BetAld, betaine aldehyde; P-Bases, phosphobases; Ptd-Bases, phosphatidylbases. The figure is adapted from McNeil *et al*. (2000) with the permission of the American Society of Plant Biologists. (b) A steady-state model used for the fluxomic analysis of the network of central metabolism in maize (*Zea mays*) root tips. Measurements included accumulation rates of starch cell wall and sucrose, and nuclear magnetic resonance (NMR) analysis of the ^{13}C labeling in different atomic positions of metabolic products after labeling to steady state with ^{13}C-glucose. Modeling of these data yielded estimates of the following flux values. *Vg*, rate of glucose uptake; *Vppp3*, fluxes catalyzed by transaldolase; *Vhk*, flux through hexokinase; *Vppp4*, fluxes catalyzed by transketolase; *Vgpase*, flux from glucose-6-phosphate (G6P) to glucose; *Valdp*, flux catalyzed by plastidic aldolase; *Vwall*, rate of wall biosynthesis; *Vpk*, oxidative flux through pyruvate kinase (PK); *Vhcp*, exchange of cytosolic and plastidic hexose-P; *Vpepc*, anaplerotic flux through phosphoenolpyruvate carboxylase; *Vgf*, fluxes catalyzed by G6P isomerase; *Vpdh*, flux catalyzed by pyruvate dehydrogenase; *Vsuc*, rate of sucrose synthesis; *Vcs*, flux through citrate synthase; *Vald*, fluxes catalyzed by aldolase; *Vca*, flux catalyzed by aconitase; *Vgly*, glycolytic flux; *Vglu*, rate of glutamate synthesis; *Vsts*, fluxes of starch synthesis and degradation; *Vsfa*, flux through 2-oxoglutarate DH; *Vsta*, rate of starch accumulation; *Vfum*, flux catalyzed by fumarase; *Vppp1*, flux of the oxidative part of the pentose-P pathway; *Vasp*, rate of aspartate production; *Vppp2*, fluxes catalyzed by transketolase; *Vme*, flux through malic enzyme. Abbreviations: GLC, glucose; GLCext, external glucose; SUC, sucrose; G6P, glucose-6-phosphate; F6P, fructose-6-phospate; H6P, hexose-6-phospate; STA, starch; TP, triose-phosphate; $P5P_P$, plastidic pentose-5-phosphate; $S7P_P$, plastidic sedoheptulose-7-phosphate; $E4P_P$, plastidic erythrose-4-phosphate; PEP, phosphoenolpyruvate; PYR, pyruvate; AcCoA, acetyl coenzyme A; CIT, citrate; OAA, oxaloacetate; FUM, fumarate; AKG, alphaketoglutarate; Glu, glutamate; Ala, alanine. From unpublished work by Ana Alonso.

The second approach, steady-state analysis, is also known as metabolic flux analysis (MFA). Here the values of metabolic and transport fluxes are derived from measurements of metabolite labeling patterns when the latter have reached stable levels (referred to as isotopic steady state). In steady-state analyses, measurements of isotopic enrichments at different atomic positions of metabolites are used to deduce flux values. No measurements of metabolite levels or estimates

of rate constants are required for steady-state analyses, nor are values for these parameters obtained. The models used in this type of analysis are based on rate equations describing metabolic transformations, but they differ from dynamic models in two important ways. First, steady-state models treat the fluxes themselves rather than the underlying metabolite pools and rate constants as the variables (parameters) to be used in the fitting process. Secondly, these models focus on metabolic branch points, with any series of reaction steps between two branch points being treated as a single entity. Accordingly, steady-state analyses can only be made of systems that are in metabolic steady state long enough to reach isotopic steady state. The flux maps obtained in these studies are less detailed than those from kinetic analyses, but the smaller number of parameters being considered makes it easier to obtain sufficient data to compute them robustly (experimental over-determination).

Steady-state fluxomic studies yield a quantitative description of the flows through a metabolic network, whereas the dynamic models yielded by kinetic studies are mechanistic. Thus, only kinetic analyses can be predictive of flux patterns under conditions other than those of the experiments used in the analysis. Another advantage of kinetic analyses is that they can be carried out when the fluxes are changing during an experiment (such as when a bolus of substrate is supplied), whereas steady-state analyses require the fluxes to remain fixed during the labeling period. Furthermore, the isotopic steady state required for steady-state analyses can take many hours or even days to be reached. These differences have thus far restricted the use of steady-state analyses of plant systems to cell cultures, isolated root tissues, and seeds developing in culture. However, steady-state studies can cover larger parts of the metabolic network and are also much better suited than dynamic studies to the analysis of central metabolism with its complex patterns of reversible, cyclic, multicompartmented fluxes.

What has fluxomics taught us about plant systems?

Figure 2 illustrates kinetic and steady-state models used in studies of plant metabolism. The flux values obtained for metabolic networks such as these constitute a flux map, and such maps have been derived by fluxomic studies for various plant systems. These range from cyanobacteria, through higher plant cell cultures, to root, leaf, flower, and seed tissues and have been reviewed in greater detail elsewhere (Kruger *et al.*, 2003; Ratcliffe & Shachar-Hill, 2006). Here, several examples of the findings made in plant fluxomic studies are described. These have been chosen for their relevance to possible future mycorrhizal investigations, the potential for which is discussed further in the following section.

Figure 2(a) describes the metabolic network involved in the synthesis of glycine betaine (an osmoprotectant; McNeil *et al.*, 2000). This model was used to pursue the rational metabolic engineering of this compound into plants that normally lack the ability to protect themselves from drought and salt stress in this manner. Using ^{33}P and ^{14}C radiolabeling experiments and kinetic modeling, McNeil *et al.* (2000) were able to identify the constraints that had hitherto limited the successful metabolic engineering of glycine betaine production into betaine nonproducing species. This research, together with a larger set of bacterial fluxomic studies, illustrates how metabolic network flux analysis can make key contributions to metabolic engineering. In particular, the ability to map fluxes of phosphorus (P) and carbon (C) units through different parts of metabolism is of direct interest to mycorrhizal research. The rational engineering of these types of metabolic and transport processes is a long-term goal of understanding mycorrhizas, and the development of predictive, mechanistic models such as that illustrated in Fig. 2(a) (McNeil *et al.*, 2000) would be a powerful aid to achieving this. Another illustrative kinetic study concerns the synthesis and emission of plant signaling compounds. In a study by Boatright *et al.* (2004), stable isotopic labeling and gas chromatography–mass spectrometry (GC-MS) measurement were used to map the pathways and dynamics by which flowers make and release volatile scent compounds. Recent progress towards discovering the plant signaling compounds involved in establishing the arbuscular mycorrhizal symbiosis (Akiyama *et al.*, 2005) suggests the possibility of using flux analysis in understanding the synthesis of such compounds.

The steady-state model shown in Fig. 2(b) covers most of central metabolism in growing root tips (Ana Alonso, unpublished work). Steady-state flux investigations of root metabolism have uncovered a high degree of metabolic inefficiency in the form of futile cycling that dissipates much of the ATP produced by respiration in this heterotrophic tissue (Dieuaide-Noubhani *et al.*, 1995; Alonso *et al.*, 2005). Cycling of carbohydrates from hexose to mannitol and back in ectomycorrhizal fungi may constitute just such a futile cycle (Martin *et al.*, 1988). The conversion of hexose to lipid and back by arbuscular mycorrhizal fungi in the symbiotic state (Bago *et al.*, 2000) results in the loss of over half the carbon involved and may in this sense be deemed a futile cycle, although it may serve an important functional role in carbon transport through the arbuscular mycorrhizal fungal mycelium.

Figure 2(b) shows the kind of flux model used in such studies to map carbon fluxes through central metabolism. The ability to map the fluxes of carbon metabolism, especially carbohydrate handling, in roots is a necessary precursor to a full understanding of carbon exchange at the plant–fungus interface of mycorrhizas. The implication of fluxomic studies on roots and other heterotrophic cells is that roots may not be carbon limited, which might be taken as support for the theory of 'luxury resource exchange' (in which plants trade surplus carbon for fungal nutrients) that was recently proposed by Kiers & Van der Heijden (2006) to form a key part of

the evolutionary basis for mutualism in the arbuscular mycorrhizal symbiosis.

In another example of what steady-state analyses of plant metabolic fluxes can reveal, Schwender *et al.* (2004b) discovered the operation of a novel metabolic route through primary metabolism in developing seeds of *Brassica napus* (canola or oilseed rape). This metabolic route involves known enzymatic reactions operating to substantially increase the efficiency with which carbon supplied by the maternal plant is used by the embryo. This example is relevant to potential investigations of mycorrhizal fungi as these have already shown the potential for just this sort of novel combination of enzymatic reactions (Bago *et al.*, 2000; Govindarajulu *et al.*, 2005).

What might network flux analysis tell us about the functioning of mycorrhizas?

The description of metabolic and transport flows through a whole network or a functional subnetwork allows one to address questions that cannot be answered at the single enzyme or even pathway level. Such questions include: 'What are the dominant routes of carbon and nitrogen flow from sources to sinks and from substrates to secondary metabolic products? What are the relative sizes of flux through alternative metabolic and transport routes? How are reductant and ATP produced and consumed during growth and development? What determines the overall efficiency of carbon utilization?' In my opinion, addressing questions of this type is central to understanding the functioning of mycorrhizal systems. Thus, a quantitative analysis of fluxes will be needed if we are to discover the contributions of different routes of P (polyphosphate vs other forms of P; fungal vs direct P uptake), nitrogen (N) (organic vs inorganic), and C (lipid vs carbohydrate) flows between and within plant roots and mycorrhizal fungi. Flux analysis is also required to address questions about mycorrhizal functioning and efficiency such as the exchange rate of C for P. Progress in such areas is necessary for understanding the ecology and evolution of mycorrhizal function (Van der Heijden & Sanders, 2002).

Experience in the last decade shows that the analysis of metabolic flux is extremely valuable in the successful modification of microbial functioning for industrial purposes (Petersen *et al.*, 2000; Dauner *et al.*, 2001). As illustrated by the studies of plant metabolism discussed above, fluxomics can contribute both to the rational design of genetic alterations and to the analysis of transgenic strains. If and when we are to successfully genetically alter the metabolic and transport characteristics of mycorrhizal fungi and their hosts to improve mycorrhizal performance in different settings, fluxomics will probably have an important part to play.

Another area in which metabolic flux analysis has the potential to contribute to mycorrhizal research in the future is that of secondary metabolism, an area that we are only beginning to explore. Experience with plant systems shows that delineation of the pathways by which metabolites involved in signaling and defense are made can be greatly facilitated by applying flux analytical methods.

The existence of *in vitro* model arbuscular and ecto mycorrhizas together with the establishment of stable isotopic and radiolabeling methods for mycorrhizal research provide the analytical tools needed for applying flux analysis approaches developed in microbes and plants to mycorrhizal research. The development of model mycorrhizas that retain the advantages of current *in vitro* model mycorrhizas but are closer to natural situations will also be important if mycorrhizal fluxomics is to be relevant to ecological and agricultural settings.

Some questions in mycorrhizal research could best be addressed using dynamic labeling approaches, where others are likely to prove more amenable to steady-state analysis. Thus studies on the routes, rates and regulation of P movement through mycorrhizal symbioses are a promising area for dynamic labeling analysis using radiolabeling and kinetic models. By contrast, steady-state analysis based on ^{13}C labeling offers the potential to significantly advance our understanding of C handling by mycorrhizal fungi and mycorrhizas, for those cases where metabolic and isotopic steady states are achievable under physiologically meaningful conditions. Another area in which dynamic labeling analysis is likely to provide valuable insights is that of N metabolism and transport. Recent results of short-term labeling with ^{15}N and mass spectrometric analysis in the arbuscular mycorrhizal symbiosis (Cruz *et al.*, 2006) show great promise for such studies.

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References

Akiyama K, Matsuzaki K, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature.* **435**: 824–827.

Alonso AP, Vigeolas H, Raymond P, Rolin D, Dieuaide-Noubhani M. 2005. A new substrate cycle in plants. Evidence for a high glucose-phosphate-to-glucose turnover from *in vivo* steady state and pulse labelling experiments with [^{13}C]-glucose and [^{14}C]-glucose. *Plant Physiology* **138**: 2220–2232.

Bago B, Pfeffer PE, Shachar-Hill Y. 2000. Carbon metabolism and transport in arbuscular mycorrhizas. *Plant Physiology* **124**: 949–957.

Boatright J, Negre F, Chen X, Kish CM, Wood B, Peel G, Orlova I, Gang D, Rhodes D, Dudareva N. 2004. Understanding *in vivo* benzenoid metabolism in petunia petal tissue. *Plant Physiology* **135**: 1993–2011.

Bucking H, Shachar-Hill Y. 2005. Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. *New Phytologist* **165**: 899–912.

Chalot M, Blaudez D, Brun A. 2006. Ammonia: a candidate for nitrogen transfer at the mycorrhizal interface. *Trends in Plant Science* **11**: 263–266.

Chalot M, Brun A. 1998. Physiology of organic nitrogen acquisition by ectomycorrhizal fungi and ectomycorrhizas. *FEMS Microbiology Reviews* **22**: 21–44.

Cruz C, Egsgaard H, Trujillo C, Ambus P, Requena N, Martins-Loução MA, Jakobsen I. 2006. Enzymatic evidence for the key role of arginine in nitrogen translocation by arbuscular mycorrhiza fungi. *Plant Physiology*, DOI: 10.1104/pp.106.090522.

Dauner M, Bailey JE, Sauer U. 2001. Metabolic flux analysis with a comprehensive isotopomer model in *Bacillus subtilis*. *Biotechnology and Bioengineering* **76**: 144–156.

Dieuaide-Noubhani M, Raffard G, Canioni P, Pradet A, Raymond P. 1995. Quantification of compartmented metabolic fluxes in maize root tips using isotope distribution from ^{13}C- or ^{14}C-labeled glucose. *Journal of Biological Chemistry* **270**: 13147–13159.

Govindarajulu M, Pfeffer PE, Jin HR, Abubaker J, Douds DD, Allen JW, Bucking H, Lammers PJ, Shachar-Hill Y. 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. *Nature* **435**: 819–823.

Jin H, Pfeffer PE, Douds DD, Piotrowski E, Lammers PJ, Shachar-Hill Y. 2005. The uptake, metabolism, transport and transfer of nitrogen in an arbuscular mycorrhizal symbiosis. *New Phytologist* **168**: 687–696.

Kiers ET, van der Heijden MGA. 2006. Mutualistic stability in the arbuscular mycorrhizal symbiosis: Exploring hypotheses of evolutionary cooperation. *Ecology* **87**: 1627–1636.

Kruger NJ, Ratcliffe RG, Roscher A. 2003. Quantitative approaches for analysing fluxes through plant metabolic networks using NMR and stable isotope labelling. *Phytochemistry Reviews* **2**: 17–30.

Martin F, Ramsted M, Soderhall K, Canet D. 1988. Carbohydrate and amino acid metabolism in the ectomycorrhizal Ascomycete *Sphaerosporella brunnea* during glucose utilization: a ^{13}C NMR study. *Plant Physiology* **86**: 935–940.

McNeil SD, Rhodes D, Russell BL, Nuccio ML, Shachar-Hill Y, Hanson AD. 2000. Metabolic modeling identifies key constraints on an engineered glycine betaine synthesis pathway in tobacco. *Plant Physiology* **124**: 153–162.

Morgan JA, Rhodes D. 2002. Mathematical modeling of plant metabolic pathways. *Metabolic Engineering* **4**: 80–89.

Petersen S, de Graaf AA, Eggeling L, Möllney M, Wiechert W, Sahm H. 2000. *In vivo* quantification of parallel and bi-directional fluxes in the anaplerosis of *Corynebacterium glutamicum*. *Journal of Biological Chemistry* **275**: 35932–35941.

Ratcliffe RG, Shachar-Hill Y. 2006. Measuring multiple fluxes through plant metabolic networks. *Plant Journal* **45**: 490–511.

Sauer U. 2004. High-throughput phenomics: experimental methods for mapping fluxomes. *Current Opinion in Biotechnology* **15**: 58–63.

Sauer U, Lasko DR, Fiaux J, Hochuli M, Glaser R, Szyperski T, Wuthrich K, Bailey JE. 1999. Metabolic flux ratio analysis of genetic and environmental modulations of *Escherichia coli* central carbon metabolism. *Journal of Bacteriology* **181**: 6679–6688.

Schwender J, Goffman F, Ohlrogge JB, Shachar-Hill Y. 2004a. Rubisco without the Calvin cycle improves the carbon efficiency of developing green seeds. *Nature* **432**: 779–782.

Schwender J, Ohlrogge J, Shachar-Hill Y. 2004b. Understanding flux in plant metabolic networks. *Current Opinion in Plant Biology* **7**: 309–317.

Van der Heijden MGA, Sanders. IR, eds. 2002. *Mycorrhizal ecology.* Berlin, Germany: Springer.

Key words: fluxomics, metabolic flux analysis, metabolic networks, mycorrhiza.

Research perspectives on functional diversity in ectomycorrhizal fungi

Communities of ectomycorrhizal fungi

We have recently witnessed an increasing number of studies of ectomycorrhizal fungal communities. This interest, in part, stems from the need to understand human impacts on the functioning of natural ecosystems and it has been facilitated by the advent of nucleic acid-based fungal detection methods. We have learned that ectomycorrhizal fungal communities are frequently species-rich, in some cases exceeding 100 taxa in relatively small plots of land (Izzo *et al.*, 2004). Most comprise few, frequently occurring species and many more rare species (Taylor, 2002; Buée *et al.*, 2005; Koide *et al.*, 2005a). Species may spatially partition the forest floor (Dickie *et al.*, 2002; Genney *et al.*, 2006) and interact with each other both positively and negatively (Agerer *et al.*, 2002; Koide *et al.*, 2005b). Moreover, the relationships between the frequency of soil hyphae and the numbers of fruiting structures and colonized roots differ markedly among species (Gardes & Bruns, 1996; Gehring *et al.*, 1998; Koide *et al.*, 2005a).

Taxonomic and functional diversity in ectomycorrhizal fungal communities

From the standpoint of ecosystem function, taxonomic diversity is only relevant insofar as it is reflective of functional diversity. For example, variation in the composition of the ectomycorrhizal fungal community on individual plants influences host growth (Jonsson *et al.*, 2001) probably because the species vary in ability to transport nutrients to the host or in their demand for carbon. This emphasis on function was reflected in the session entitled 'Functional diversity in mycorrhiza' at the last International Conference on Mycorrhiza (Granada, July 2006). Because we feel, as did Bengtsson (1998), that there is more utility in understanding the relationship between ecosystem functions and species traits than between ecosystem functions and taxonomic diversity *per se* (about which there has been much debate), our purpose here is to highlight some methods that can be used to document functional variability among species of ectomycorrhizal fungi, as well as to discuss briefly the utility in doing so. It seems reasonable to concentrate on functions that influence the success of both the fungi and their hosts. Thus, functions relating to the acquisition of water, carbon (C), phosphorus (P) and nitrogen (N), and the exchange of resources between plants and fungi may be among the most relevant.

