



This is a repository copy of *Nitrogen transport in the orchid mycorrhizal symbiosis - further evidence for a mutualistic association.*

White Rose Research Online URL for this paper:  
<http://eprints.whiterose.ac.uk/110365/>

Version: Accepted Version

---

**Article:**

Dearnaley, J.D.W. and Cameron, D.D. [orcid.org/0000-0002-5439-6544](https://orcid.org/0000-0002-5439-6544) (2016) Nitrogen transport in the orchid mycorrhizal symbiosis - further evidence for a mutualistic association. *New Phytologist*, 213 (1). pp. 10-12. ISSN 0028-646X

<https://doi.org/10.1111/nph.14357>

---

This is the peer reviewed version of the following article: Dearnaley, J. D. W. and Cameron, D. D. (2017), Nitrogen transport in the orchid mycorrhizal symbiosis – further evidence for a mutualistic association. *New Phytol*, 213: 10–12, which has been published in final form at <https://doi.org/10.1111/nph.14357>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

**Reuse**

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

**Takedown**

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing [eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk) including the URL of the record and the reason for the withdrawal request.



[eprints@whiterose.ac.uk](mailto:eprints@whiterose.ac.uk)  
<https://eprints.whiterose.ac.uk/>

## Commentary

### Nitrogen transport in the orchid mycorrhizal symbiosis – further evidence for a mutualistic association

#### Introduction

Mycorrhizas are symbioses integral to the health of plant-based ecosystems (Smith & Read, 2008). In a typical mycorrhizal association, fungi in or on plant roots pass soil-acquired inorganic nutrients and water to the plant host. In return, the host transfers excess photosynthate to the fungus. Orchid mycorrhizas were considered to be unusual symbioses in that during initial colonisation of the young, non-photosynthetic host, the fungus was thought to provide both inorganic and organic nutrition to the plant and to receive nothing in return for its services. That is until a significant new study by Fochi et al. (New Phytologist, this issue) investigating expression of fungal and plant nitrogen (N) transport and assimilation genes in mycorrhizas formed between the fungus *Tulasnella calospora* and the photosynthetic orchid, *Serapias vomeracea*. The research suggests, for the first time, flow of nutrients back to the fungal partner from the non-photosynthetic orchid host. Thus orchid mycorrhizas now appear to represent a true mutualism in both the early and mature stages of plant development (Cameron et al., 2006) and they thus join the physiological ranks of the more extensively studied arbuscular mycorrhizal and ectomycorrhizal associations.

The Orchidaceae constitutes the largest of all plant families with estimates of more than 27,000 species (The Plant List, 2013). Orchids are typically pollinated by insects, with most having quite specific associations which can impact on rarity (Phillips et al., 2011). The dust-like seed released from orchid fruits is wind dispersed to locations away from the parent plant. At this stage, a compatible mycorrhizal fungus, usually basidiomycete members of the Cantharellales and Sebaciniales (Dearnaley et al., 2012) grow into the tissues of the ungerminated seed or a pre-seedling stage known as a protocorm. Fungal coils or pelotons are produced inside the cells of the orchid by the mycorrhizal fungus (Fig. 1). Nutrients such as carbon (C), phosphorus (P) and N are transferred dynamically from the mycorrhizal fungus across the interfacial matrix, a new apoplastic space produced between the fungal peloton and the orchid cell membrane (Kuga et al., 2014; Fig. 1). Fungal nutrients such as P, N and C also pass to the orchid from rupturing hyphae as the pelotons are digested by the host after a few days (Fig. 1). As the plant becomes photosynthetic, plant to fungus C flow becomes established (Cameron et al., 2006; Fig. 1) finalising the life-stage dependent trophic switch from heterotrophic juvenile to autotrophic adult.

Nitrogen can be a limiting element in natural and agricultural ecosystems (Gress et al., 2007). Organisms require N for protein and nucleic acid synthesis but also to manufacture a wide array of coenzymes. Plants may obtain N via direct uptake of nitrate from soils. Leguminous plants use bacteria in nodules to acquire amides and ureides. Many plants also use mycorrhizal fungi to take up N (Thirkell et al., 2016). In arbuscular mycorrhizas, fungi transfer ammonium to the host plant in exchange for photosynthate (Koegel et al., 2015). In ectomycorrhizas, both organic and inorganic N forms appear to be transported to the plant host (reviewed in Muller et al., 2007). Fungi are well known to transfer N to plants in orchid mycorrhizas and this possibly includes both organic and inorganic forms (Cameron et al., 2006; Kuga et al., 2014). Mycorrhizal fungi will target environmental N sources as demonstrated by in vitro growth experiments (Leigh et al., 2008).

### **The common orchid mycorrhizal fungus, *Tulasnella calospora*, cannot use nitrate**

*Tulasnella calospora* is a cosmopolitan orchid mycorrhizal fungus, colonising many photosynthetic species in Europe, North America, South America, Australia and Asia (reviewed in Dearnaley, 2007). Fochi et al. cultured *T. calospora* on a variety of media containing different sources of N. As evidenced by comparisons of mycelial dry weight, the fungus grew best on organic N sources such as glutamine and glutamic acid and ammonium-based media but grew poorly on media containing sodium nitrate. Searches of the *T. calospora* genomic database identified two functional ammonium transporters but no nitrate uptake and assimilation genes. Heterologous expression of these ammonium transporter cDNAs in yeast showed that they encoded functional genes. Using RNA-Seq analysis, one of the ammonium transporters, TcAMT2 was significantly upregulated in free living mycelium growing on ammonium. The discovery that *T. calospora* is unable to utilise environmental nitrate is interesting from a number of perspectives. Threatened orchids which exclusively associate with *T. calospora* eg. *Diurus*, *Drakaea* and *Thelymitra* spp. (Warcup, 1981; Nurfadilah et al., 2013) will need appropriate N sources in ex situ growth media and in soils that are used for restoration work. The basis for some orchids to become weeds can perhaps be explained by a capacity to form mycorrhizal associations with fungi in addition to *Tulasnella calospora* (Bonnardeaux et al., 2007), which in turn may belie a capacity to access multiple soil N sources.

### **Molecular evidence that *T. calospora* transports amino acids to orchids**

Cameron et al., (2006) was first to suggest via  $^{13}\text{C}$ - $^{15}\text{N}$  isotope tracing experiments that the mycorrhizal fungus, *Ceratobasidium cornigerum* transfers organic N to orchid hosts. Contrastingly, a recent study by Kuga et al. (2014), utilising cellular level imaging of stable isotope tracers suggested that  $^{15}\text{NH}_4^{15}\text{NO}_3$  passed from mycorrhizal fungus to orchid across intact pelotons. Fochi et al. investigated fungal and plant amino acid transport genes in free-living mycelium of *T. calospora* and asymbiotic and symbiotic protocorms of *S. vomeracea*. These investigations showed that a number of fungal amino acid transporters/permeases (TcAAT1, TcAAT2, TcAAT6) were significantly upregulated in the symbiotic situation. RT-PCR analyses of laser microdissected protocorm cells showed the presence of TcAAT1 transcripts in peloton-containing cells. Upregulation of plant amino acid transporters such as the permeases, SvAAP1 and SvAAP2 and a lysine-histidine transporter was shown in symbiotic protocorms. Fungal to orchid transfer of amino acids was also strongly suggested by assessment of fungal N assimilation gene expression. In symbiotic protocorms, fungal arginase and urease, enzymes involved in amino acid breakdown, were only weakly expressed. Additionally, a fungal argininosuccinate lyase, an enzyme involved in arginine biosynthesis, was upregulated in symbiosis. Thus orchids largely appear to acquire N in an organic form from their fungal partner. Further assessment of fungal to orchid inorganic N transfer is however, needed in light of the findings of Kuga et al. (2014).

### **Why do mycorrhizal fungi colonise the seeds and protocorms of orchids?**

The research presented by Fochi et al. also provides a new perspective on orchid to fungal nutrient transport. In 2006, Cameron et al. significantly changed the landscape of orchid mycorrhizal physiology when it was shown, using  $^{14}\text{CO}_2$  tracer experiments, that the photosynthetic orchid *Goodyera repens* transfers approximately 3% of fixed C to its fungal partner. For decades it was thought that early, non-photosynthetic orchid stages provided little reward for their fungal partners. Indeed, Leake et al. (2008) suggested that the impetus for fungal colonisation of non-photosynthetic plant tissues involved the concept of “give now but get more later”, in that the C invested in orchid seedlings would be returned with interest from photosynthetic adults. In their study, Fochi et al. demonstrated upregulation of the plant ammonium transporter SvAMT1 in symbiotic protocorms of *Serapias vomeracea*. TcAMT2, a fungal ammonium transporter was also significantly upregulated in symbiosis and transcripts of both TcAMT2 and TcAMT1 could be found in laser microdissected peloton-containing cells. That the orchid passes ammonium back to its fungal partner in the *T.*

calospora-S. vomeracea system was also suggested by symbiotic upregulation of the fungal TcGS1 glutamine synthetase - an enzyme which scavenges ammonium to use in the synthesis of glutamine. These findings also cast light on how a compatible mycorrhizal fungus might detect an orchid seed or protocorm in soil. Orchid seeds have few reserves apart from lipid stores and protein bodies (Rasmussen, 1995). Following seed imbibition, these latter structures could potentially be catalysed to release ammonium into the environment and entice fungal colonisation.

### **A new model for nutrient transport in orchid mycorrhizas**

In view of the study by Fochi et al. and other key work by researchers in the field (eg. Cameron et al., 2006; 2007; Bougoure et al., 2013; Kuga et al., 2014), a new model for nutrient transport in orchid mycorrhizas can be proposed (Fig. 1). In non-photosynthetic orchid stages such as seeds and protocorms, the plant exports  $\text{NH}_4^+$  (Fochi et al.) and receives P, N and C (the latter two nutrients as fungal amino acids) from the fungal partner across intact membranes (Cameron et al., 2006; 2007; Kuga et al., 2014; Fochi et al.). Lysis of fungal pelotons also releases P, N and C to the plant (Bougoure et al., 2013). In photosynthetic orchids, the plant exports sugars to the fungus (Cameron et al., 2006) and receives only P from the fungal partner across intact membranes as fungus to plant, amino acid transport at this location discontinues in photosynthetic orchids (Cameron unpublished). Fungal P, N and C from lysed hyphae continues to be taken up by the mature orchid host.

### **Implications of bidirectional nutrient transfer in young and adult orchids**

The research of Fochi et al. suggests that orchid mycorrhizas represent a true mutualism in that both symbionts benefit at all stages of the association. Such a finding reinforces an old ecological adage, in that in nature there is “no such thing as a free lunch” and thus orchid seeds and protocorms must pay for the nutrients that the fungal partner provides. As the orchid mycorrhizal system is easy to study in vitro (ie. both symbionts are axenically culturable), this suggests that it could provide a useful model system to investigate biological aspects of the more difficult to manipulate, arbuscular and ectomycorrhizal associations. Continued investigations of orchid mycorrhizas may assist with unravelling the intricacies of other mycorrhizal systems and ultimately improve the health and sustainability of natural, horticultural and agricultural systems.

### **John D.W. Dearnaley\***

Faculty of Health, Engineering & Sciences  
University of Southern Queensland  
Toowoomba, Australia, 4350

(\*Author for correspondence: tel 61 7 4631 2804; email john.dearnaley@usq.edu.au)

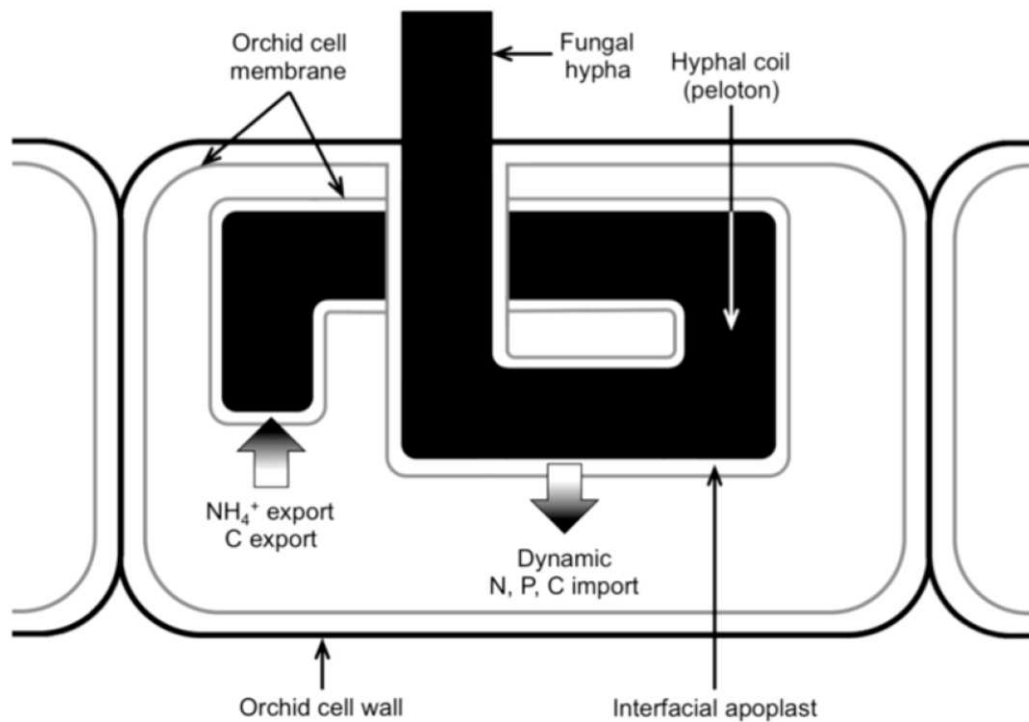
### **Duncan D. Cameron**

Department of Animal & Plant Sciences,  
University of Sheffield,  
Alfred Denny Building  
Western Bank, Sheffield, S10, 2TN, UK

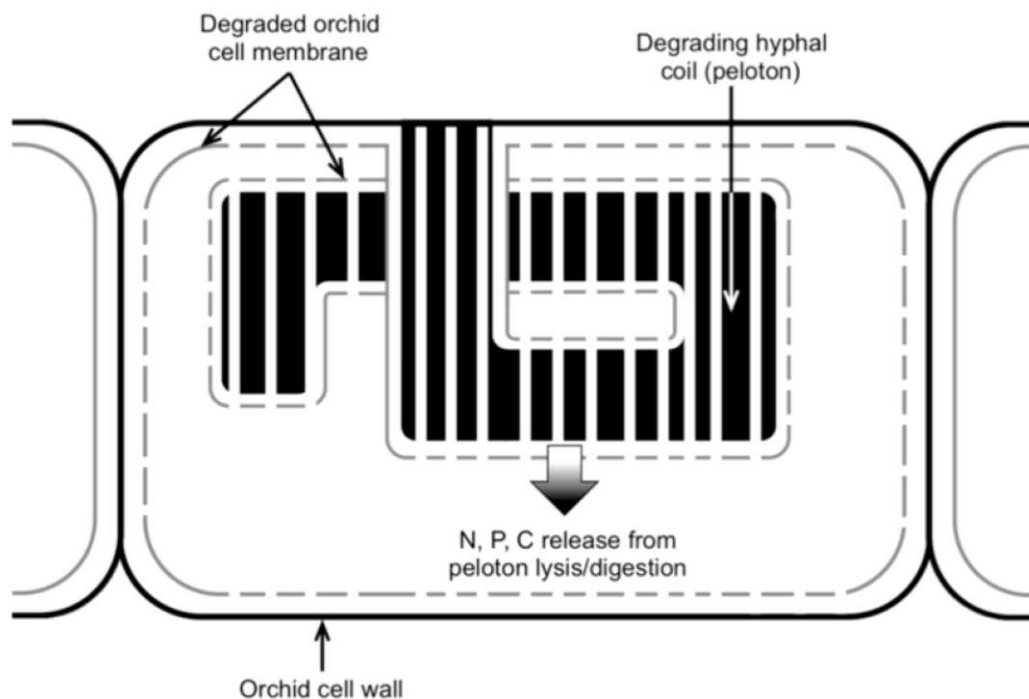
## References

- Bonnardeaux Y, Brundrett M, Batty A, Dixon K, Koch J, Sivasithamparam K. 2007.** Diversity of mycorrhizal fungi of terrestrial orchids: compatibility webs, brief encounters, lasting relationships and alien invasions. *Mycological Research* **111**: 51-61.
- Bougoure J, Ludwig M, Brundrett M, Cliff J, Clode P, Kilburn M, Grierson P. 2013.** High-resolution secondary ion mass spectrometry analysis of carbon dynamics in mycorrhizas formed by an obligately myco-heterotrophic orchid. *Plant, Cell and Environment* **39**: 1123-1230.
- Cameron DD, Leake JR, Read DJ. 2006.** Mutualistic mycorrhiza in orchids: evidence from plant-fungus carbon and nitrogen transfers in the green-leaved terrestrial orchid *Goodyera repens*. *New Phytologist*. **171**: 405-416.
- Cameron DD, Johnson I, Leake JR, Read DJ. 2007.** Mycorrhizal acquisition of inorganic phosphorus by the green-leaved terrestrial orchid *Goodyera repens*. *Annals of Botany* **99**: 831-834.
- Dearnaley JDW. 2007.** Further advances in orchid mycorrhizal research. *Mycorrhiza*. **17**: 475-486.
- Dearnaley JDW, Martos F, Selosse M-A. 2012.** Orchid mycorrhizas: molecular ecology, physiology, evolution and conservation aspects. In: Esser K, Ed. *The Mycota Volume IX - Fungal Associations*, 2nd ed., Berlin: Springer-Verlag, 207-230.
- Gress SE, Nichols TD, Northcraft CC, PeterJohn WT. 2007.** Nutrient limitation in soils exhibiting differing nitrogen availabilities: what lies beyond nitrogen saturation? *Ecology* **88**: 119-130.
- Koegel S, Brule D, Wiemken A, Boller T, Courty PE. 2015.** The effect of different nitrogen sources on the symbiotic interaction between *Sorghum bicolor* and *Glomus intradices*: expression of plant and fungal genes involved in nitrogen assimilation. *Soil Biology and Biochemistry* **86**: 156-163.
- Kuga U, Sakamoto N, Yurimoto H. 2014.** Stable isotope imaging reveals that both live and degenerating fungal pelotons transfer carbon and nitrogen to orchid protocorms. *New Phytologist* **202**: 594-605.
- Leake JR, Cameron DD, Beerling SJ. 2008.** Fungal fidelity in the myco-heterotroph-to-autotroph lifecycle of *Lycopodiaceae*: a case of parental nurture? *New Phytologist* **177**: 572-576.
- Leigh, J, Hodge A, Fitter AH. 2008.** Arbuscular mycorrhizal fungi can transfer substantial amounts of nitrogen to their host plant from organic material. *New Phytologist* **181**: 199-207.
- Muller T, Avolio M, Olivi M, Benidia M, Rikirsch E, Kasaras A, Fitz M, Chalot M, Wipf D. 2007.** Nitrogen transport in the ectomycorrhiza association: The *Hebeloma cylindrosporum* – *Pinus pinaster* model. *Phytochemistry* **68**: 41-51.
- Nurafadilah S, Swarts ND, Dixon KW, Lambers H, Merritt DJ. 2013.** Variation in nutrient acquisition patterns by mycorrhizal fungi of rare and common orchids explains diversification in a global biodiversity hotspot. *Annals of Botany* **111**: 1233-1241.
- Phillips RD, Barrett MD, Dixon KW, Hopper SD. 2011.** Do mycorrhizal symbioses cause rarity in orchids? *Journal of Ecology* **99**: 858-869.
- Rasmussen HN. 1995.** *Terrestrial orchids from seed to mycotrophic plant*. Cambridge: Cambridge University Press.
- Smith SE, Read DJ. 2008.** *Mycorrhizal symbiosis*. Cambridge: Academic Press.
- The Plant List. 2013.** The plant list, Version 1. <http://www.theplantlist.org>. Accessed 20<sup>th</sup> September 2016.
- Thirkell TJ, Cameron DD, Hodge A. 2016.** Resolving the “nitrogen paradox” of arbuscular mycorrhizas: fertilisation with organic matter brings considerable benefits for plant nutrition and growth. *Plant, Cell and Environment* **39**: 1683-1690.
- Warcup JH. 1981.** Specificity of mycorrhizal associations in some Australian terrestrial orchids. *New Phytologist* **70**: 41-46.

a) Intact, mature orchid peloton cell



b) Senescent, degrading orchid peloton cell



**Fig. 1** Model of nutrient transport in orchid mycorrhizas. a) In orchid cells containing intact, mature pelotons, the plant exports  $\text{NH}_4^+$  to the fungus in non-photosynthetic stages and C in photosynthetic stages. The plant imports N, P & C from the fungus across intact membranes. b) In orchid cells containing senescent pelotons, the plant receives N, P and C as the hyphal coils are digested.