THE ROYAL SOCIETY

doi 10.1098/rspb.2001.1681

Exploitation of pollen by mycorrhizal mycelial systems with special reference to nutrient recycling in boreal forests

J. Perez-Moreno† and D. J. Read*

Department of Animal and Plant Sciences, University of Sheffield, Western Bank, Sheffield S10 2TN, UK

Very large quantities of pollen are released annually by wind-pollinated trees, which dominate northern forest ecosystems. Since pollen is enriched in both nitrogen and phosphorus, this recurrent pulse of deposition constitutes a significant potential source of these elements in what are known to be severely nutrient-limited systems. Here, we demonstrate for the first time, to our knowledge, that an ectomycorrhizal fungus, *Paxillus involutus*, is able to scavenge effectively for nitrogen and phosphorus in pollen and to return a significant proportion of each nutrient to its autotrophic host, *Betula pendula*. More than 75 and 96%, respectively, of the nitrogen and phosphorus were removed from pollen in microcosms containing the mycorrhizal fungus, 29 and 25%, respectively, being transferred to the plants. In contrast, in microcosms without the mycorrhizal fungus only 42 and 35%, respectively, of nitrogen and phosphorus were lost from the pollen, presumably as a result of export by saprotrophs, and only 12 and 7%, respectively, were transferred to the plants. We hypothesize that this process of resource recapture, by contributing significantly to the ability of the trees to sustain the necessary annual investment in pollen production, will have a major impact upon their reproductive capabilities and hence 'fitness'.

Keywords: nutrient mobilization; vegetative mycelium; ectomycorrhizal fungi; organic substrates; pine forests; pollen

1. INTRODUCTION

An enormous quantity of pollen is produced annually by the anemophilous trees, such as pine and birch, that dominate boreal forests, only a small fraction of which becomes involved in the reproductive process. Conservative estimates of the quantity of pollen released from mature pine forests range from 10–80 kg ha^{-1} yr^{-1} (Koski 1970; Eriksson *et al*. 1995; Lee *et al*. 1996), while values as high as 1–3 tonnes ha^{-1} yr^{-1} have been reported (Greenfield 1999). Though adapted for long-range dispersal, most pollen grains are deposited within a short distance from their site of production (Koski 1970; Richards 1986; Faegri *et al*. 1989; Frankel & Galun 1997), where they constitute a significant potential source of the major nutrient elements nitrogen and phosphorus, which often limit productivity in boreal- and temperate-forest systems. The nitrogen content of pollen is between 2 and 3% (Lee *et al*. 1996; Greenfield 1999), while the phosphorus content is *ca*. 0.4% (Oleksyn *et al*. 1999). These values equate to 1.6 kg ha^{-1} yr^{-1} of nitrogen and 0.32 kg ha^{-1} yr^{-1} of phosphorus if the deposition rate is 80 kg ha^{-1} yr^{-1}, or to 60 kg ha^{-1} yr^{-1} and 12 kg ha^{-1} yr^{-1} of nitrogen and phosphorus, respectively, if production is as high as 3 tonnes ha yr^{-1}. Fractionation of pollen nitrogen (Greenfield 1999) has revealed that only a small proportion (*ca*. 5%) is recalcitrant; the bulk consists of α-amino-nitrogen (*ca*. 45%) and hydrolizable polymeric nitrogen (*ca*. 45%), most of the latter being in the form of protein and nucleic acid. It is likely that a significant proportion of the phosphorus content of pollen will also be in organic form, membrane and nucleic-acid components being important in this case.

Despite awareness of the nutrient impoverishment that characterizes boreal ecosystems, rather little thought has so far been given to the processes whereby this annual pulse of nutrient input might be accessed and redistributed. Stark (1972) hypothesized that the pollen 'rain' would constitute a significant seasonal source of nutrients, especially nitrogen and phosphorus, for litter decay fungi. In a subsequent test of this hypothesis, Hutchison & Barron (1997) confirmed that hyphae of 41 out of the 147 fungal species tested would penetrate pollen grains on agar plates, ramify through their interiors and consume the contents. They, like Stark, emphasized the potential importance of pollen as a nitrogen source for litter-decomposing fungi. However, of even greater interest at the ecosystem level is the question of the extent to which the resources contained in the pollen can be recovered by the trees that invested so heavily in their production.

Recent studies of the nutrition of mycorrhizal plants have revealed that the extensive ectomycorrhizal mycelial network, which interconnects trees in nature (Simard *et al*. 1997; Read 1997), the so-called 'wood-wide web' (Helgason *et al*. 1998; Sen 2000), plays a key role in the mobilization and transport of mineral nutrients to the tree roots (Smith & Read 1997). In addition, it has recently been demonstrated that the extraradical mycelium growing from the mycorrhizal roots is involved in selective exploitation of natural substrates and subsequently in the mobilization and transfer of nutrients from these organic materials to the associated trees (Bending & Read 1995; Perez-Moreno & Read 2000). However, the notion that the mycorrhizal fungal symbionts might also have access to the nutrients contained in pollen, thereby enabling direct feedback to

*Author for correspondence (d.j.read@sheffield.ac.uk).
† Permanent address: Microbiologia, Edafologia-IRENAT, Colegio de Postgraduados, Montecillo, Edo. de Mexico, CP 56230, Mexico.

Received 27 October 2000 *Accepted* 26 March 2001

the trees, has received little attention. While Raidl (1997) showed that the hyphae of some ectomycorrhizal fungi are able to penetrate pollen grains, there have been no measurements of nitrogen and phosphorus mobilization by mycorrhizal systems.

Here, by growing birch (*Betula pendula* Roth) in association with *Paxillus involutus* (Fr.) Fr., which is one of the most widespread ectomycorrhizal symbionts of anemophilous trees in northern Europe (Laiho 1970; Atkinson 1992), and supplying pollen as the only major source of both macronutrients, we test the hypothesis that a mycorrhizal symbiont can enhance the ability of trees to recover significant proportions of the nitrogen and phosphorus that were originally invested in pollen production.

2. MATERIAL AND METHODS

Seedlings of birch (*B. pendula*) were grown in mycorrhizal or non-mycorrhizal conditions in microcosms containing pollen as the only significant potential source of nutrients. *Betula* was used as the assay genus because, in addition to its ecological importance as a component of forests throughout the boreal and tundral zones of the Northern hemisphere, birch seeds are among the smallest and least nutrient-enriched of all tree seeds. This leads them to become dependant upon and responsive to exogenous sources of nutrients very soon after germination, as well as enabling accurate determination and budgeting of the nutrients acquired. The pollen used was that of *Pinus sylvestris* L., which is a frequent co-associate of *Betula* in Eurasian forests and which, therefore, contributes significantly to the pollen rain in many *Betula* habitats. The advantage, in addition to ecological relevance, of using *Pinus* pollen as a substrate is that the literature provides detailed quantitative and qualitative information concerning the production, nutrient dynamics, deposition and post-depositional fractionation of pollen released by this genus (Koski 1970; Greenfield 1999; Oleksyn *et al.* 1999).

Seeds of *B. pendula* were surface sterilized by immersion in H_2O_2 for 25 min, then rinsed with sterile distilled water and placed to germinate on water agar. *P. involutus* (strain USPI97) was used as the fungal symbiont. This culture was originally isolated from a fruit body growing under *Betula* in Cropton Forest, North Yorkshire, UK, but *P. involutus* has a wide host range and is one of the dominant ectomycorrhizal fungi of birch and pine forests in Eurasia (Laiho 1970). Mycorrhizae were synthesized on a subset of seedlings using a procedure adapted from Brun *et al.* (1995). *P. involutus* was first grown on cellophane discs overlying very dilute nutrient agar with a pH adjusted to 5.7, in 9 cm Petri dishes. The seedlings were aseptically transferred to the dishes so that their roots were in contact with the mycelium as it spread across the disc. Mycorrhiza formation commenced within 24 h of transfer and all lateral roots were colonized within 14 days. At this stage, these seedlings, and a corresponding subset of non-mycorrhizal plants, were transferred singly into microcosms with or without pollen as the only major nutrient source.

Microcosms were constructed as described by Perez-Moreno & Read (2000), using square transparent plastic sheets (15 cm × 15 cm). Over the surface of the lower plate a film of water agar was poured, onto which was spread a thin layer of *Sphagnum* peat over inert clay (leca) beads. These layers provided a moist nutrient-poor matrix for support of the seedlings and, in the case of mycorrhizal plants, for growth of the ectomycorrhizal mycelium. Pollen was added in small (3 cm × 3 cm) plastic trays to half the mycorrhizal and half the non-mycorrhizal microcosms. An equal number of microcosms lacking pollen were set up. Each tray contained, as a support matrix, 2 g dry weight of clay granules in 2 ml of dilute (0.8%) agar, onto the surface of which was spread 200 mg of freshly collected pollen. Two pollen-containing trays (i.e. 400 mg of pollen in total) were added per chamber, together with a single tray containing support matrix only, the latter being used to provide a comparison of mycelial development in trays with and without pollen. This quantity of pollen was equivalent to a deposition of 178 kg ha^{-1} which, while being somewhat greater than that observed by Koski (1970), was considerably lower than the 1000–3000 kg ha^{-1} reported by Greenfield (1999), and can therefore be considered to be within the range likely to be encountered in nature.

There were three replicate microcosms with pollen (designated + pollen) and three without pollen (designated − pollen) in both the mycorrhizal and the non-mycorrhizal treatments. Representative + pollen and − pollen mycorrhizal microcosms at time zero are shown in figure 1*a*,*c*. All microcosms were incubated in a controlled-environment growth cabinet (day–night temperature 15–10 °C, 18 L:6 D photoperiod, irradiance 150 μmol m^{-2} s^{-1}). Dry weights and total nitrogen and phosphorus contents of seeds, plants and pollen were determined at time zero, at the time of transfer of seedlings and pollen to microcosms and at the final harvest, which was carried out 115 days after pollen addition. At this point, 83 and 87% of root tips supported *Paxillus* mycorrhizae in plants grown in microcosms with and without pollen, respectively, there being no significant difference between these values according to a Student's *t*-test ($p < 0.05$). The plants were partitioned into roots and shoots, and oven-dried at 80 °C before dry weights were measured and determinations of their tissue nitrogen and phosphorus contents were carried out.

3. RESULTS

Because of their extremely small size, *Betula* seeds contain only minute quantities of nitrogen and phosphorus (table 1). There was some gain of these elements by both mycorrhizal and non-mycorrhizal seedlings during the period of mycorrhiza synthesis (table 1), but at the time of their transfer to the microcosms there were no significant differences between the nitrogen and phosphorus contents of the two categories of plant.

Within one week of the transfer of mycorrhizal seedlings to the microcosms, hyphae of *P. involutus* had grown from the colonized roots into the surrounding soil and advanced towards the pollen-containing trays. In the case of mycorrhizal + pollen microcosms, contact of the mycelial front with the pollen was followed by the intensive proliferation of its constituent hyphae, which, over the ensuing 115 days, formed a progressively larger mat over the surface (figure 1*b*). In the same period, the roots of the seedlings in the non-mycorrhizal + pollen systems grew weakly through the surrounding matrix and failed to make contact with the pollen. Following contact between *P. involutus* and the pollen, the shoots of mycorrhizal seedlings increased progressively in vigour. Visual observations revealed continuous production of new leaves in mycorrhizal plants in + pollen microcosms, a situation that contrasted strongly with that in − pollen microcosms of both mycorrhizal and non-mycorrhizal

Table 1. *Dry weights* (mg) *and total nitrogen and phosphorus contents* (μg) *of seeds and plants of* Betula pendula *grown in microcosms for 115 days, either in the mycorrhizal condition with the fungus* Paxillus involutus *or as non-mycorrhizal plants, in the presence and absence of pollen*

(Values are given as mean ± 95% confidence intervals of three to six replicates. Asterisks indicate significant differences between nutrients in microcosms with mycorrhizal and non-mycorrhizal plants; and between seed and seedling after mycorrhiza synthesis using *t*-tests at $^{*}p < 0.05$ and $^{**}p < 0.01$.)

treatment	dry weight (mg)	nitrogen content (μg)	phosphorus content (μg)
seed	0.2 ± 0.1	5.3 ± 1.3	0.6 ± 0.1
seedling after mycorrhiza synthesis	3.7 ± 2.0**	54.6 ± 30.4**	6.2 ± 3.8*
microcosms after 115 days with pollen			
non-mycorrhizal plants	150.3 ± 48.7	1994.6 ± 717.5	106.7 ± 41.9
mycorrhizal plants	931.6 ± 442.5*	4432.4 ± 1825.5*	411.6 ± 210.9*
microcosms after 115 days without pollen			
non-mycorrhizal plants	61.2 ± 34.7	302.2 ± 290.5	13.2 ± 15.7
mycorrhizal plants	112.1 ± 33.8*	366.5 ± 103.0	68.0 ± 18.0**

plants, where leaves failed to develop. Root growth followed a very similar pattern (figure 1*b*,*d*).

By the final harvest, both root and shoot dry weights of mycorrhizal and non-mycorrhizal plants of + pollen microcosms were significantly greater than those of the corresponding − pollen microcosms, but the differences between the + pollen and − pollen treatments were much greater in the case of mycorrhizal plants (figure 2*a*,*b*). In + pollen microcosms, shoot and root dry weights of mycorrhizal plants were 3.8 and 9.9 times, respectively, greater than those of their non-mycorrhizal counterparts (figure 2*a*,*b*). By harvest, mycorrhizal seedlings in + pollen microcosms had achieved total dry weights 8.3 times greater than those of their counterparts in microcosms lacking pollen. The biomass of mycorrhizal + pollen plants was 6.2 and 15.2 times greater, respectively, than those of plants in the two non-mycorrhizal treatments (non-mycorrhizal + pollen and non-mycorrhizal − pollen) (table 1).

Analyses of the nitrogen and phosphorus contents of these plants revealed a similar overall pattern (figure 2*c*–*f*). In all cases, except for the nitrogen content of roots in the non-mycorrhizal treatment, plants grown with pollen contained significantly more nitrogen and phosphorus than those grown without pollen. Again, however, in the mycorrhizal microcosms, the differences between + pollen and − pollen treatments were much greater than in microcosms that lacked mycorrhizae. The nitrogen contents of roots and shoots (figure 2*c*,*d*) were 4.7 and 1.2 times greater, respectively, in mycorrhizal than in non-mycorrhizal plants supplied with pollen. In the case of phosphorus (figure 2*e*,*f*), these differences were, 6.5 and 2.8 times, respectively.

The yield increases and gains of nutrients by both mycorrhizal and non-mycorrhizal plants in + pollen microcosms were associated with a significant depletion of nutrients in pollen ($p < 0.05$) (figure 3). However, the mycorrhizal systems mobilized a significantly greater amount of both nitrogen and phosphorus than the non-mycorrhizal systems, the biggest difference being seen in the case of phosphorus. The reductions in nitrogen and phosphorus concentrations in mycorrhizal systems were 1.8 and 2.8 times, respectively, greater than those in non-mycorrhizal systems, there being significant differences for both nutrients ($p < 0.05$) (figure 3). Colonization of pollen by the mycorrhizal fungus led to a reduction in total nitrogen and phosphorus contents after 115 days of 75.8 and 96.9%, respectively, there being a highly significant difference ($p < 0.001$) between these and the non-mycorrhizal systems, where the depletions of the same nutrients were 41.6 and 34.5%, respectively (table 2). In the non-mycorrhizal − pollen treatment, plants showed increases in nitrogen content that were not significantly different from those seen in the equivalent mycorrhizal treatment. However, these non-mycorrhizal plants had considerably lower phosphorus contents, indicating that mycorrhizal colonization increased the ability of *Betula* plants to scavenge for phosphorus in the surrounding peat (table 1).

Comparisons of nutrient-gain values in mycorrhizal and non-mycorrhizal systems indicate that the symbiotic systems are more efficient in nutrient transfer to the plants: the total nitrogen and phosphorus transfers from pollen were 2.4 and 3.7 times, respectively, greater in mycorrhizal plants than in non-mycorrhizal plants (table 2). Since this transfer represents only a portion of the nutrients mobilized from the pollen, it can be concluded that the remaining portion is retained by the fungal mycelium. In the longer term, some of this may also be released to the plant.

4. DISCUSSION

Analyses of nitrogen and phosphorus contents of pollen and plants showed that in the presence of mycorrhizal mycelium there was a significant mobilization of both macronutrients. Previous studies of mobilization of nutrients by ectomycorrhizal mycelial systems from organic natural materials (Entry *et al*. 1991; Bending & Read 1995; Perez-Moreno & Read 2000) have employed chemically ill-defined substrates such as litter from the 'F' horizon of forest soil. Nutrient translocation from such residues by different ectomycorrhizal species has been reported, in these studies, to range from 0 to 32% and from 3 to 40% for nitrogen and phosphorus, respectively. In contrast, the quantities of these elements removed from pollen, in the present study, amounting to 76% for nitrogen and 97% for phosphorus, were much greater.

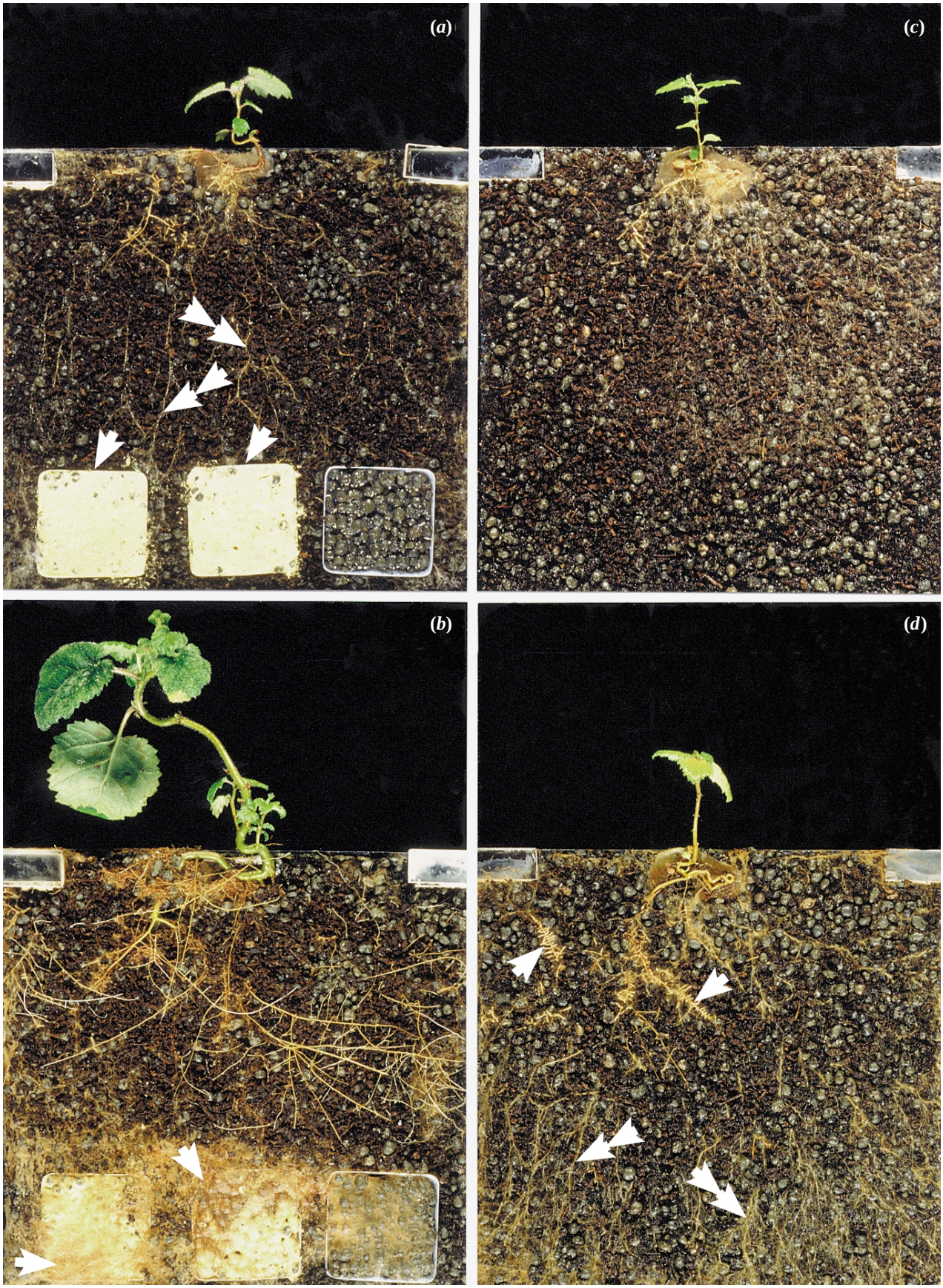

Figure 1. Microcosms supporting *Betula* plants grown in mycorrhizal association with *Paxillus involutus*. (*a*) At the time of addition of pollen trays (single arrow heads), showing the mycelial network surrounding the trays (double arrow heads). The third tray contains support matrix only. (*b*) The same microcosm after 115 days, showing invigoration of the mycelium over and around the pollen trays (single arrow heads) and the large growth response of the plant. (*c*) Microcosm at the same stage as in (*a*) but to which no pollen was added. (*d*) The microcosm in (*c*) after 115 days. Note the differentiated mycelial network (double arrow heads) and extensive mycorrhizal development (single arrow heads) but the lack of plant development.

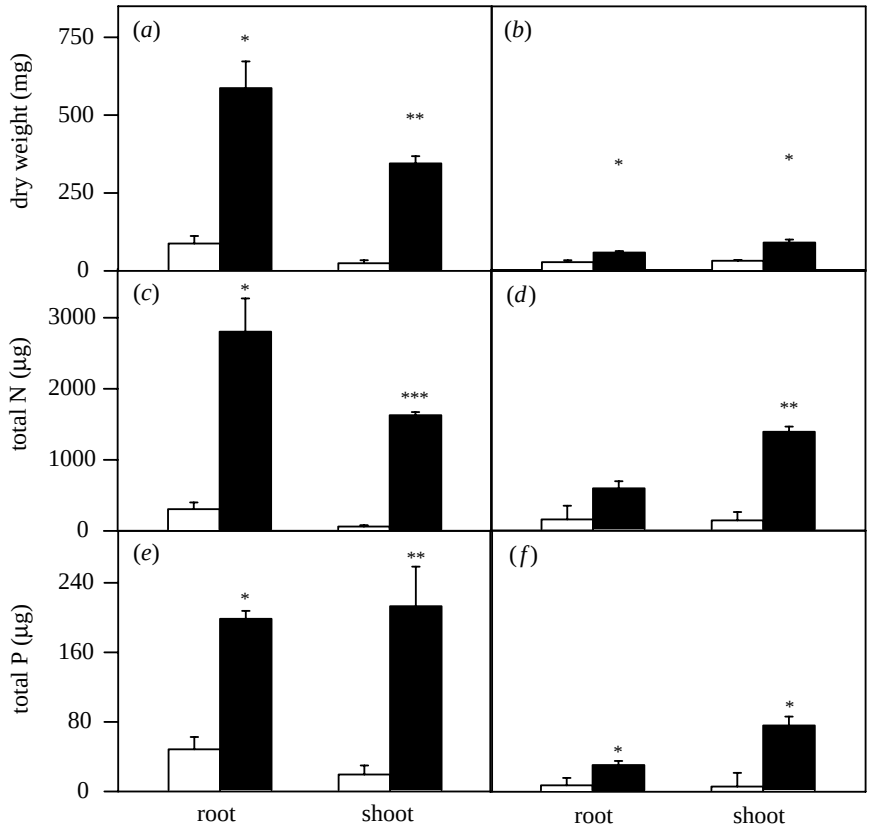

Figure 2. Dry-weight yields and nitrogen and phosphorus contents of mycorrhizal (*a*,*c*,*e*) and non-mycorrhizal (*b*,*d*,*f*) plants grown for 115 days in microcosms with (closed bars) and without (open bars) pollen as a potential nutrient source. Values are expressed as means (±s.e.m.). Asterisks indicate significant differences within tissue category according to Student's *t*-test: $^{*}p < 0.05$; $^{**}p < 0.005$; $^{***}p < 0.001$.

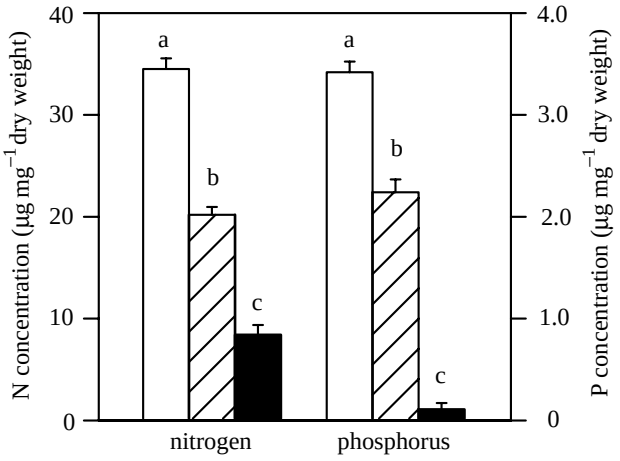

Figure 3. Concentrations of nitrogen and phosphorus in pollen at the time of addition to the microcosms (open bars) and after 115 days in microcosms containing non-mycorrhizal plants (hatched bars) and plants colonized by the mycorrhizal fungus *Paxillus involutus* (closed bars). Values are expressed as means (+s.e.m.). Within nutrients, a change of letter over a bar indicates a significant difference in concentration of the element according to Tukey's multiple-comparison test ($p < 0.05$).

Clearly, over the period of the experiment, not all of the nitrogen and phosphorus removed from the pollen was transferred to the plant. Calculations show (table 2) that 29% of the nitrogen and 25% of the phosphorus reached the plant by the time of harvest. The remaining 46 and 72% of the total pollen nitrogen and phosphorus, respectively, was presumed to have been retained by the mycelium of *P. involutus*. Using a similar microcosm approach, Perez-Moreno & Read (2000) found that, on average, 5 and 13%, respectively, of the nitrogen and phosphorus originally present in three types of litter were transferred to the associated mycorrhizal plants.

Our results confirm the view that saprotrophs have some ability both to absorb and to export a component of the soluble nitrogen and phosphorus contained in pollen. In microcosms with non-mycorrhizal plants, 12 and 7% of the nitrogen and phosphorus, respectively, originally present in pollen was transferred to the plants. This transfer may have been facilitated by saprotrophic fungi growing from the pollen into the surrounding medium. This pathway for redistribution of the resources provides an indirect and clearly relatively inefficient mechanism of nutrient transfer to the plant. It is apparent from our study that the mycorrhizal mycelial network, supported by assimilates obtained from the autotroph, will be the major sink for both elements, and this provides a direct pathway for the transfer of the essential nutrients to the plant. Thus, while the nutrient fund contained in pollen may indeed contribute to the maintenance of decomposer activities, the largest proportion is effectively recycled to the trees by the mycorrhizal mycelium. Neither Stark (1972) nor Hutchison & Barron (1997) considered the possibility that ectomycorrhizal fungi could be recipients of the nutrients contained in pollen, yet it is evident from

Table 2. *Nitrogen and phosphorus budgets of pollen and plants of* Betula pendula *grown with the mycorrhizal fungus* Paxillus involutus *or in the non-mycorrhizal condition for 115 days*

(Values are means ± 95% confidence intervals of three to six replicates. Asterisks indicate significant differences between nutrients in microcosms with mycorrhizal and non-mycorrhizal plants using t-tests at $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$.)

	total nutrient contents (μg) of added pollen		reduction of nutrients in pollen in relation to initial contents		gain of nutrients by plants[a] in relation to initial contents in pollen	
nutrient and treatment	initial	after 115 days	μg	%	μg	%
nitrogen						
microcosms with non-mycorrhizal plants	13824.6 ± 1083.5	8072.9 ± 802.0	5751.7 ± 477.5	41.6 ± 2.3	1692.4 ± 436.0	12.2 ± 3.2
microcosms with mycorrhizal plants	13824.6 ± 1083.5	3364.0 ± 667.0***	10460.6 ± 557.0***	75.8 ± 3.1***	4065.9 ± 1723.5*	29.4 ± 12.5*
phosphorus						
microcosms with non-mycorrhizal plants	1367.2 ± 72.3	895.7 ± 131.5	471.6 ± 62.3	34.5 ± 6.3	93.5 ± 26.2	6.8 ± 1.9
microcosms with mycorrhizal plants	1367.2 ± 72.3	42.8 ± 14.6***	1324.4 ± 59.6***	96.9 ± 0.9***	343.6 ± 193.6*	25.1 ± 14.2*

[a]The gain of nutrients by plants was calculated from the values in table 1. Gain in mycorrhizal plants = mycorrhizal plants with pollen - mycorrhizal plants without pollen; and gain in non-mycorrhizal plants = non-mycorrhizal plants with pollen - non-mycorrhizal plants without pollen.

the present work that a very large proportion of the nitrogen and phosphorus contained in the pollen supplied to the microcosms was not only absorbed by these fungi but also returned by them to the plants.

It is necessary to consider the extent to which the processes of nutrient mobilization and transfer observed in the microcosms reflect those likely to occur in nature. In boreal forest regions, pollen, most of which falls to the ground within a few metres of its site of production (Koski 1970; Richards 1986; Faegri *et al.* 1989; Frankel & Galun 1997), will first contact the litter layer and the soil surface. Here, saprotrophic fungi are, indeed, likely to be the dominant component of the microflora, and the proportion of the labile material that is lost from pollen at this stage will depend on its residence time at the soil surface. It appears, however, that the residence time is relatively short, since Dimbleby (1957) observed that, in woodland, pollen grains become concentrated immediately below the soil surface, and Stark (1972) refers specifically to their accumulation in the 'F' layer. Stark (1972) also observed that pollen grains in this position were 'attacked by at least two species of hyphal fungi', which she believed to be saprotrophic.

Awareness that this horizon is occupied by ectomycorrhizal fungi and that their mycelium has the phenotypic plasticity to respond rapidly to the presence of nutrient-enriched substrates (Entry *et al.* 1991; Read 1992; Bending & Read 1995; Perez-Moreno & Read 2000) has developed only recently. While it can be envisaged that there will be some competition between saprotrophic and mycorrhizal fungi for the nitrogen and phosphorus contained in this resource, ultimately, access to carbon in the form of photosynthate is likely to give symbionts the advantage in such interactions. Even in the litter layer, any nutrients acquired by saprotrophs immediately after pollen deposition will presumably be returned as the 'pulse' of input ends and carbon limitation once again sets in.

Whatever the first sink for the nutrients contained in pollen may be, the issue of their final destination is of primary interest from the ecosystem perspective. Our results strongly suggest that investment by the plant in the carbon that sustains the mycorrhizal mycelial network will enable its fungal symbiont to recover a high proportion of the nitrogen and phosphorus originally invested in pollen production. Since these are acknowledged to be the key growth-limiting nutrients in the boreal forest, their recovery could be of vital importance not only for growth but also for sustained reproductive activity. The annual cycle of pollen production and release, upon which reproduction depends, must pose a considerable recurrent nutritional burden upon the trees, and their ability to recover a major proportion of these resources is likely to contribute to the sustainability of both their photosynthetic and sexual activities. While in terms of ecosystem productivity the former may be of primary importance, in evolutionary terms any contribution of this kind to the effectiveness of the reproductive process can be seen to be linked, in a Darwinian sense, directly to the 'fitness' (Fisher 1958) of the pollen-producing plants. The greater vigour observed in the vegetative mycorrhizal mycelium growing in the + pollen microcosms indicates that feedback to the fungal symbiont, presumably in the form of enhanced supply of photosynthate, is a further effect of pollen exploitation. This suggests that the annual input of pollen may lead to increases in fitness of both partners in the symbiosis.

We thank Consejo Nacional de Ciencia y Tecnología, Colegio de Postgraduados (Mexico) and the British Chevening Scholarships programme for financial support to J.P.-M., and we acknowledge the skilled technical assistance of I. Johnson and G. Woods.

REFERENCES

Atkinson, M. D. 1992 *Betula pendula* Roth (*B. verrucosa* Ehrh.) and *B. pubescens* Ehrh. *J. Ecol.* **80**, 837–870.

Bending, G. D. & Read, D. J. 1995 The structure and function of the vegetative mycelium of ectomycorrhizal plants. V. Foraging behavior and translocation of nutrients from exploited litter. *New Phytol.* **130**, 401–409.

Brun, A., Chalot, M., Finlay, R. D. & Söderström, B. 1995 Structure and function of the ectomycorrhizal association between *Paxillus involutus* (Batsch) Fr. and *Betula pendula* Roth. I. Dynamics of mycorrhiza formation. *New Phytol.* **129**, 487–493.

Dimbleby, G. W. 1957 Pollen analysis of terrestrial soils. *New Phytol.* **56**, 12–28.

Entry, J. A., Rose, C. L., Cromack Jr, K. 1991 Litter decomposition and nutrient release in ectomycorrhizal mat soils of a Douglas fir ecosystem. *Soil Biol. Biochem.* **23**, 285–290.

Eriksson, U., Jansson, G., Yazdani, R. & Wilhelmsson, L. 1995 Effects of supplemental mass pollination (SMP) in a young and a mature seed orchard of *Pinus sylvestris*. *Tree Physiol.* **15**, 519–526.

Faegri, K., Kaland, P. E. & Krzywinski, K. 1989 *Text book of pollen analysis*, 4th edn. London: Wiley.

Fisher, R. A. (ed.) 1958 *The genetical theory of natural selection*, revised edn. New York: Dover Press.

Frankel, R. & Galun, E. 1997 *Pollination mechanisms, reproduction and plant breeding.* Berlin: Springer.

Greenfield, L. G. 1999 Weight loss and release of mineral nitrogen from decomposing pollen. *Soil Biol. Biochem.* **31**, 353–361.

Helgason, T., Daniell, T. J., Husband, R., Fitter, A. H. & Young, S. P. W. 1998 Ploughing up the wood-wide web? *Nature* **394**, 431.

Hutchison, L. J. & Barron, G. L. 1997 Parasitism of pollen as a nutritional source for lignicolous Basidiomycota and other fungi. *Mycol. Res.* **101**, 191–194.

Koski, V. 1970 A study of pollen dispersal as a mechanism of gene flow in conifers. *Comm. Inst. Forest. Fenn.* **70**, 1–78.

Laiho, O. 1970 *Paxillus involutus* as a mycorrhizal symbiont of forest trees. *Acta Forest. Fenn.* **106**, 1–65.

Lee, J., Kenkel, T. & Booth, T. 1996 Atmospheric deposition of macronutrients by pollen in the boreal forest. *EcoScience* **3**, 304–309.

Oleksyn, J., Reich, P. B., Karolewski, P., Tjoelker, M. G. & Chalupka, W. 1999 Nutritional status of pollen and needles of diverse *Pinus sylvestris* populations grown at sites with contrasting pollution. *Water Air Soil Poll.* **110**, 195–212.

Perez-Moreno, J. & Read, D. J. 2000 Mobilization and transfer of nutrients from litter to tree seedlings via the vegetative mycelium of ectomycorrhizal plants. *New Phytol.* **145**, 301–309.

Raidl, S. 1997 Studien zur Ontogenie an Rhizomorphen von Ektomykorrhizen. *Bibl. Mycol.* **169**, 1–184.

Read, D. J. 1992 The mycorrhizal mycelium. In *Mycorrhizal mycelium: an integrative plant fungal process* (ed. M. J. Allen), pp. 102–133. New York: Chapman & Hall.

Read, D. J. 1997 The ties that bind. *Nature* **388**, 517–518.

Richards, A. J. 1986 *Plant breeding systems*. London: George Allen & Unwin.

Sen, R. 2000 Budgeting for the wood-wide web. *New Phytol.* **145**, 161–163.

Simard, S. W., Perry, D. A., Jones, M. D., Myrold, D. D., Durall, D. M. & Molina, R. 1997 Net transfer of carbon between ectomycorrhizal tree species in the field. *Nature* **388**, 579–582.

Smith, S. E. & Read, D. J. 1997 *Mycorrhizal symbiosis*, 2nd edn. San Diego, CA: Academic Press.

Stark, N. 1972 Nutrient cycling pathways and litter fungi. *BioScience* **22**, 355–360.