

## Chapter (non-refereed)

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# Mixtures and mycorrhizas: the manipulation of nutrient cycling in forestry

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## 13.1 Introduction

Although, in agriculture, soil fertility is primarily maintained by frequent fertilizer applications, this is not usually the case with forestry. The nutrients required by forest trees are normally provided by nutrient cycling, which is the circulation of nutrients in the forest ecosystem. It is only where this supply is seriously inhibited that repeated fertilizer applications are required. The increasing cost of fertilizers and their potentially environmentally undesirable effects, described by Hornung and Adamson (see page 55), may make their use in forestry even less attractive in future. Thus, it is important to see if we can, where necessary, boost fertility levels by enhancing nutrient cycling instead.

This paper describes two aspects of research which are based in Cumbria, at the Merlewood Research Station of the Institute of Terrestrial Ecology (ITE), but which have a very wide application. Historically, much of Merlewood's research has centred on the cycling of nutrients through woodland ecosystems. In the present paper, we aim to show the practical relevance of some of this work, by indicating how the forest manager may be able to manipulate nutrient cycling to improve plantation production.

A high proportion of the nutrients taken up by trees end up in the foliage, where they service photosynthesis and other physiological activities. Following leaf-fall, cycling of the nutrients remaining in the leaf litter depends on two main processes:

- i. the release of nutrients by the decomposer activities of various organisms in the soil and litter (of trees and other plants): the adjacent planting of two different species of trees (tree mixtures) appears to enhance these activities.
- ii. the ability of the trees to locate and take up these nutrients: manipulation of mycorrhizas may enhance this process. Mycorrhizas are an intimate symbiotic relationship between plant roots and fungi which are common on the majority of higher plants throughout the world.

## 13.2 Tree mixtures

Although there has been a recent renewal of interest in the possible benefits of tree mixtures, the idea that one species benefits from the presence of an admixed 'nurse' species is an old one. What sorts of mixtures lead to these benefits? Do all mixtures have this

effect? What processes and mechanisms are involved?

The joint ITE/Forestry Commission mixtures and monocultures experiment at Gisburn (Forestry Commission's Bowland Forest in north-west England) provides some answers to these questions. Established in 1955, it contains four tree species – Scots pine (*Pinus sylvestris*), Norway spruce (*Picea abies*), sessile oak (*Quercus petraea*) and alder (*Alnus glutinosa*) – planted both as monocultures and as all possible two-species mixtures. The resulting ten treatments are replicated three times in 0.2 ha plots (Brown & Harrison 1983). The site has never been fertilized.

Height measurements of each species, repeated at intervals since planting, show that several different sorts of mixture effect occur; not all combinations are beneficial in terms of overall growth of the mixture. Data for the most recent measurements (age 26 years) are given in Table 1 (see also Lines 1982).

Table 1. Dominant heights (m) of four tree species at 26 years when grown pure and mixed: Gisburn, 1981. Heights in italics are for pure stands. Data for a given measured species (ie within columns) with different suffix letters (a, b, c, d) are significantly different at  $P < 0.001$ , except for differences between spruce-with-alder and spruce-with-pine in which  $P < 0.01$

| In mixture with | Measured species   |                   |                   |       |
|-----------------|--------------------|-------------------|-------------------|-------|
|                 | Spruce             | Oak               | Alder             | Pine  |
| Spruce          | 8.80 <sup>a</sup>  | 5.67 <sup>a</sup> | 7.57 <sup>a</sup> | 11.54 |
| Oak             | 8.76 <sup>a</sup>  | 6.58 <sup>b</sup> | 7.72 <sup>a</sup> | 11.34 |
| Alder           | 9.84 <sup>b</sup>  | 7.29 <sup>c</sup> | 8.24 <sup>b</sup> | 11.12 |
| Pine            | 10.62 <sup>c</sup> | 8.82 <sup>d</sup> | 9.31 <sup>c</sup> | 11.12 |

NOTE: Some of these *plot* differences, although statistically significant as indicated, cannot strictly be ascribed to *treatment* effects because of the small number of replicates (blocks). Only differences associated with the beneficial effect of pine are rigorously referable to a treatment effect

Admixed pine stimulates height growth of all three other species without detriment to its own performance, whilst alder, although enhancing growth of oak and spruce, does so only at the expense of its own height growth. When oak and spruce are in mixture together, each grows worse than when grown separately.

Studies at Gisburn into the mechanisms involved in the mixture effects have been confined to the influence of admixed species on spruce. To identify whether improved height growth of spruce in the mixed stands was caused by improved nutrition, foliar analysis of the spruce, in pure and mixed stands, was carried out for nitrogen (N), phosphorus (P) and potassium (K), the three nutrients most commonly in short supply in forests. Results correspond with findings from other sites (O'Carroll 1978; McIntosh & Tabbush 1981; Taylor 1985), namely that N nutrition is a significant factor in the mixture effect. The improved height growth of spruce, in mixture with pine and alder, was associated with the highest levels of foliar N; the poorer growth of the pure spruce and of spruce admixed with oak was reflected in lower N concentrations (Figure 1). In addition, our results indicate that the bet-

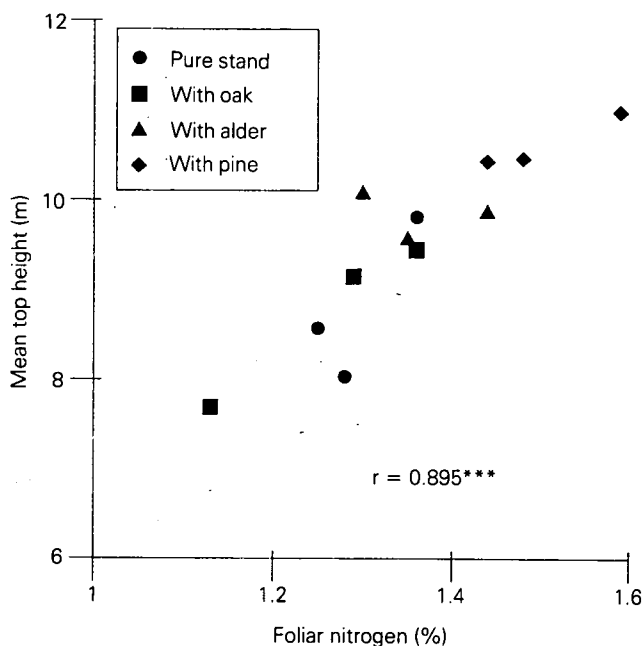


Figure 1. Relationship between tree heights and foliar nitrogen (means per plot for each of three blocks): Gisburn, 1981

ter growth is also associated with higher foliar P (Figure 2). On the other hand, there was no relationship between foliar K and spruce heights.

In the case of the spruce trees which benefit from the presence of pine and alder, where does the extra N and P come from? The fact that the nutritional benefit appears to be confined to these two elements suggests an organic matter source. As the rate of breakdown of such material is known to limit cycling of these two nutrients more than any others, an estimate

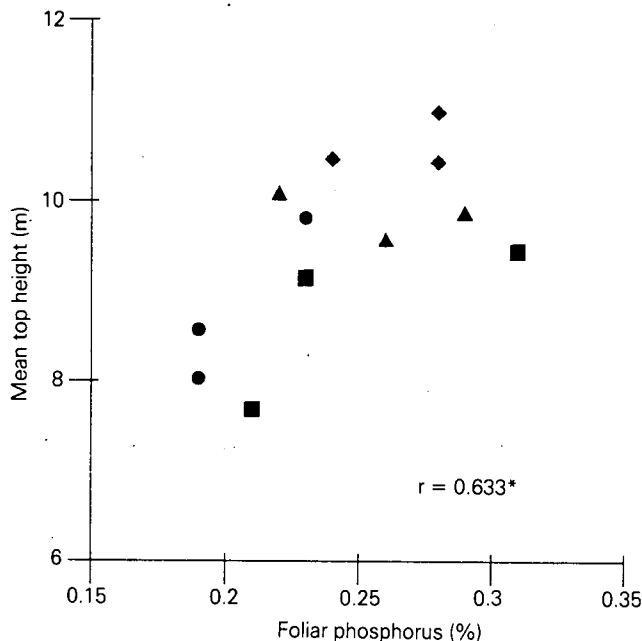


Figure 2. Relationships between tree heights and foliar phosphorus (means per plot for each of three blocks): Gisburn, 1981. Symbols as Figure 1

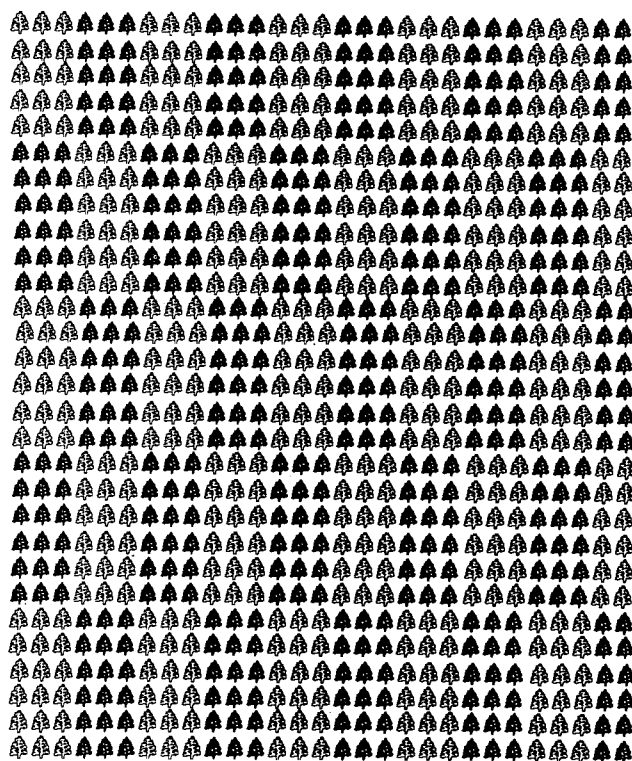


Figure 3. Layout of a mixed plot at Gisburn. Away from the edge of the plot each species is arranged in alternating blocks of three trees by six trees

was made of whether the biological activity which is responsible for decomposition was different in the pure and mixed stands of spruce.

The mixed plots at Gisburn (Figure 3) consist, for the most part, of groups of 18 trees of one species alternating in each direction with similar groups of the other species. Hence, it is possible to sample the soil within a spruce mini-plot, beneath a spruce canopy, both in the pure and mixed stands. The activity of both earthworms and decomposer micro-organisms are important in organic matter turnover, and hence in the release of the nutrients contained within such material. Both have been studied within the spruce mini-plots of the mixtures, and in monocultures, and both showed appreciable (and statistically significant) effects of mixtures. Figure 4 indicates the greatly increased pre-

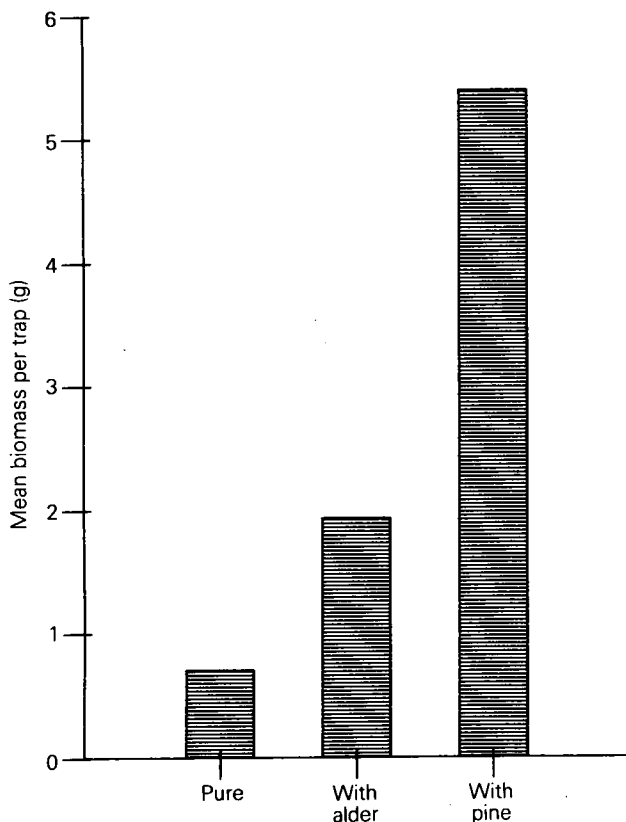


Figure 4. Mean biomass of earthworms per trap, in pure and mixed spruce: Gisburn, 1981, Blocks II and III combined

sence of earthworms within the spruce parts of the mixed stands. Microbial activity was measured indirectly by determining the degree of rotting of buried

pieces of a standard cotton cloth; this is an index of decomposer potential (Latter & Howson 1977). Cotton is a cellulosic material, and acts as a standardized and convenient analogue of forest litter. In this method, the degree of rotting is measured, after retrieval, by testing for loss in tensile strength. Compared with pure spruce, the soils of the spruce mini-plots in the mixed plots had a greater capacity to rot cotton cloth (Figure 5) (see also Brown & Howson 1988). From

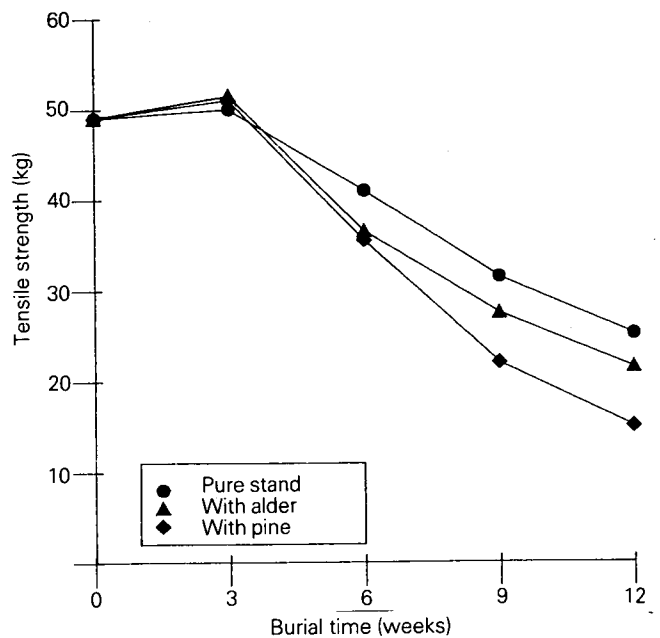


Figure 5. Changes with time in tensile strength of buried cotton cloth under spruce (pure and mixed): Gisburn, 1981, all depths and blocks combined

these results, it may be inferred that the increased biological activity in mixed stands has broken down the organic matter more quickly, releasing more N and P. In confirmation of this view, preliminary measurements of extractable nitrate-N – again from under the spruce portions of the mixed stands – showed increased levels where alder is present, and very marked increases in the pine mixtures. Similarly, use of a method to assess the availability of P, using radioactively labelled P, showed a close parallel between available P and available nitrate (Brown & Harrison 1983).

All the evidence, therefore, supports the view that the presence of the 'nurse' species stimulates biological activity and nutrient availability in a way not yet fully

understood: in other words, it enhances nutrient recycling, and hence crop growth. That the effect is sufficiently marked to obviate the need for fertilizer applications has been shown very clearly by some Forestry Commission experiments (Taylor 1985), the results from one of which are presented in Figure 6. At

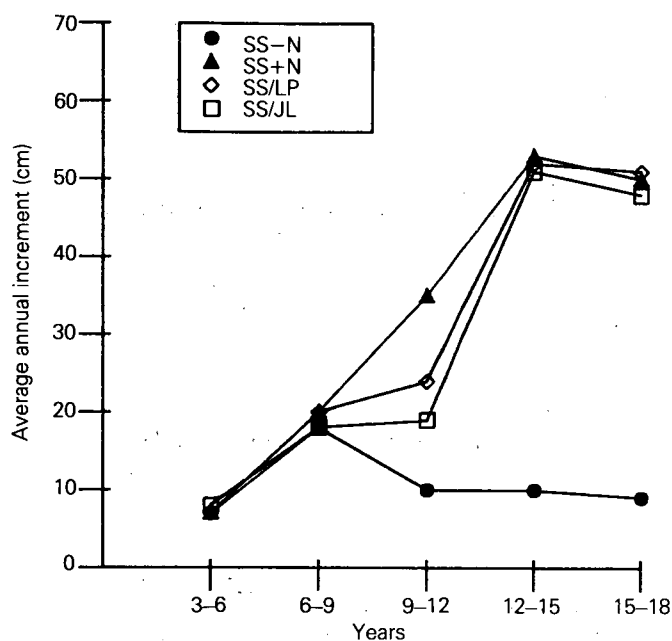


Figure 6. Height growth of Sitka spruce planted on deep peat at Inchnacardoch, Scotland (Taylor 1985)

SS-N Pure Sitka spruce without nitrogen fertilizer

SS+N Pure Sitka spruce with three-yearly nitrogen fertilizer applications

SS/LP Sitka spruce in mixture with lodgepole pine (no nitrogen fertilizer)

SS/JL Sitka spruce in mixture with Japanese larch (no nitrogen fertilizer)

this site, availability of nitrogen was so low that adequate growth could only be achieved in pure Sitka spruce (*Picea sitchensis*) by repeated (three-yearly) additions of nitrogenous fertilizer. On the other hand, admixed lodgepole pine (*Pinus contorta*) or Japanese larch (*Larix kaempferi*) had fully substituted for the fertilizer additions by years 12–15.

Although not all combinations of mixtures are equally beneficial, there may often be circumstances where appropriate mixtures could be beneficial – even on sites such as Gisburn which are not markedly nutrient deficient. Judging from the known examples of suc-

cessful mixtures, we should perhaps expect the best effects when a pioneer tree species provides the 'nurse' for a more demanding species.

### 13.3 Mycorrhizas

Can we also alter the ability of the tree roots to absorb nutrients by manipulating their mycorrhizas?

We are concerned, chiefly, with the ecto- or sheathing mycorrhizas of trees. These usually involve the association of a toadstool-forming fungus (Basidiomycotina) and the root, where the fungus forms a layer or sheath around the fine roots and penetrates between the cells of the cortex. There are benefits of the association to both partners: the tree provides carbohydrates for fungal growth, and the fungus increases the nutrient capture capabilities of the tree root system. In many cases, this symbiosis can result in a tree seedling growing faster and having a higher nutrient content than a similar seedling growing in the absence of the fungus. If selection of fungi and artificial inoculation of 'efficient' strains or species could be increased to a commercial scale, this increased growth and improved nutritional status could be exhibited in increased forest yield. How can a selection of the fungus be made? In essence, there are two potential ways in which this can be done. First, we may set up large-scale trials, using known host tree species in combination with as many fungal species and strains as we can isolate and grow in culture. With thousands of fungal species and a number of strains per species it would be an overwhelming task. The second approach would be to narrow down this choice, already reduced somewhat by those fungi we are not yet capable of growing in artificial media, by investigating the ecology and ecophysiology of the more common fungal symbionts we encounter.

One of the interesting points regarding mycorrhizal development on trees is that the populations of fungi associated with the roots change with increasing age of the tree (Mason *et al.* 1982). This change has been noted for silver birch (*Betula pendula*), planted on ex-agricultural land in trial plots, where observation over ten years has shown that genera such as *Hebeloma*, *Laccaria* and *Inocybe* are dominant on young trees, while *Cortinarius*, *Russula* and *Amanita* are dominant on older trees. Similar changes in population are found in plantation forests of Sitka spruce and lodgepole pine where stands of differing age were compared (Dighton, Poskitt & Howard 1986). Here again, genera such

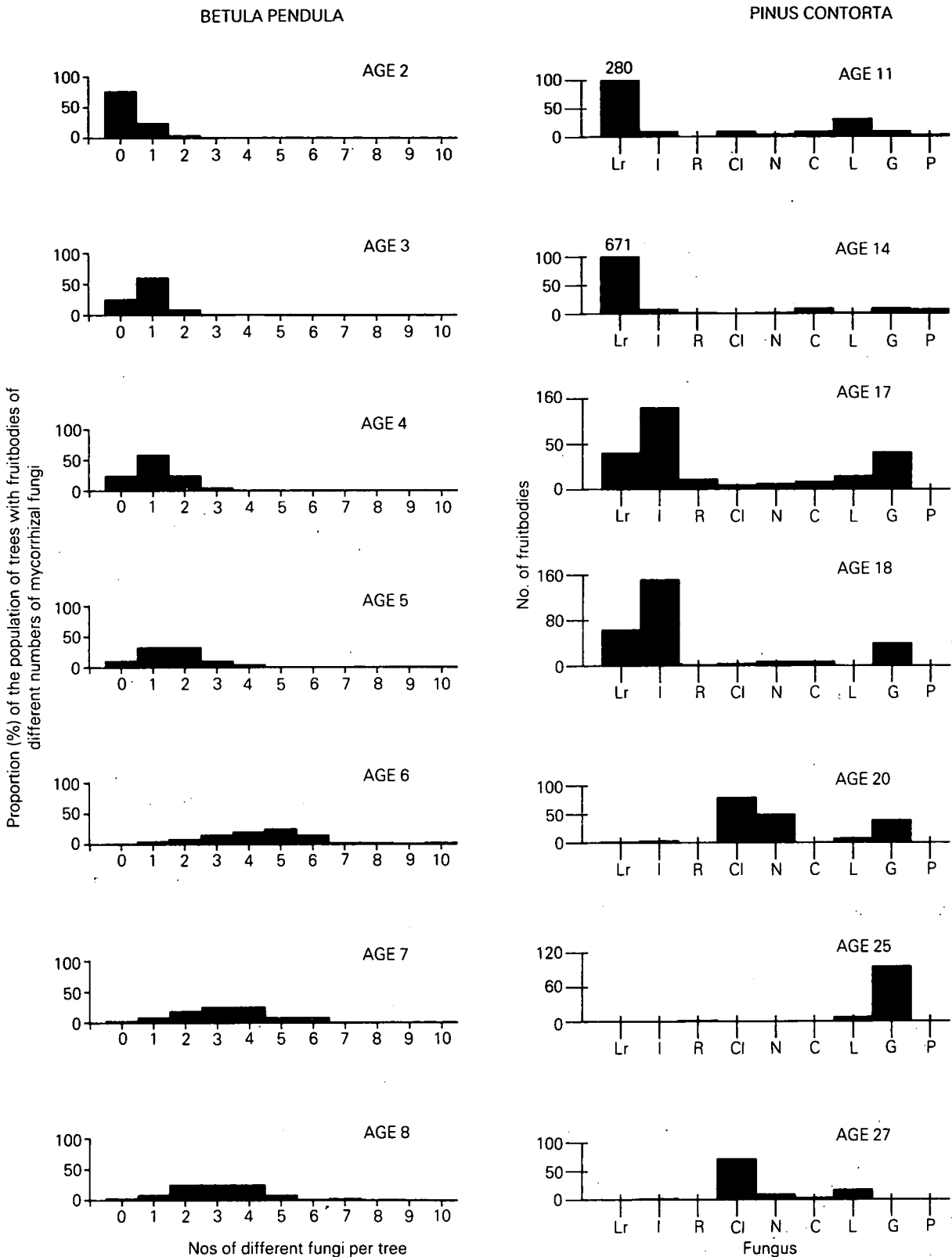


Figure 7. Changes in dominance of mycorrhizal fungal partner with increasing age of silver birch and lodgepole pine stands, based on observations of fruitbodies (Mason et al. 1982; Dighton, Poskitt & Howard 1986)

Lr = *Lactarius rufus*                      R = *Russula emetica*                      L = *Laccaria spp.* } mycorrhizal  
 I = *Inocybe longicystis*                  C = *Cortinarius croceofolius*              P = *Paxillus involutus* } fungi  
 Cl = *Clitocybe sp.*  
 N = *Nolanea cetrata*  
 G = *Galerina sp.*

as *Laccaria* and *Lactarius* were found in young stands, a wider spectrum of species of *Lactarius*, *Inocybe* and *Cortinarius* in stands at canopy closure, and a reduced flora of *Russula* and *Paxillus* in older, mature stands (Figure 7). The successions we have described are not unique to the UK; similar trends having been shown in France and New Zealand, and they suggest that some common underlying process drives this succession (Chu-Chou 1979; Chu-Chou & Grace 1981; Mosse, Stribley & Le Tacom 1981). If the succession is dependent on soil or tree factors which alter during forest stand growth, then it is important, for potential manipulation of the mycorrhizal flora, to select fungi from appropriate stages of the succession (ie it may not be practicable or suitable to use a 'late-stage' fungus on a young transplant tree).

What evidence do we have that there might be changes in the physiology of mycorrhizal fungi from different stages in the succession which would affect their efficacy? Evidence comes to us from a number of different lines of work looking at different mycorrhizal species. This evidence is far from complete and does not encompass the entire range of potential fungi by any means. One piece of evidence suggests that the tree's supply of carbohydrate can influence the fungal partner with which it can associate (Dighton & Mason 1985). 'Early-stage' fungi are able to grow on media containing low levels of carbohydrate, as would be expected to be available from small trees. Later-stage fungi have a much greater demand for carbohydrates which, it is assumed, can be supplied from a larger tree (Table 2). Mycorrhizal fungi also vary in their ability

Table 2. Growth of mycorrhizal fungi at three glucose levels (number of crosses is arbitrary scale of growth from 1 to 4 based on colony diameter on agar)

|                              | Glucose level         |                       |                      |
|------------------------------|-----------------------|-----------------------|----------------------|
|                              | 0.1 g l <sup>-1</sup> | 1.0 g l <sup>-1</sup> | 10 g l <sup>-1</sup> |
| Early <i>Hebeloma</i> spp.   | ++++                  | ++                    | +                    |
| <i>Leccinum</i> spp.         | +                     | ++                    | +++                  |
| Late <i>Amanita muscaria</i> | +                     | ++                    | +++(+)               |

to supply nutrients to their tree host. In an experiment with mycorrhizal lodgepole pine seedlings, radioactively labelled phosphorus was supplied to the peat in which they were growing. The four different mycorrhizal fungi caused different rates of incorporation of phosphorus into the plant tissues, with two of the

fungi giving lower tissue concentrations than non-mycorrhizal seedlings and two giving greater tissue concentrations (Figure 8). Such differences imply considerable variability between fungal symbionts, in extracting nutrients from soil solution.

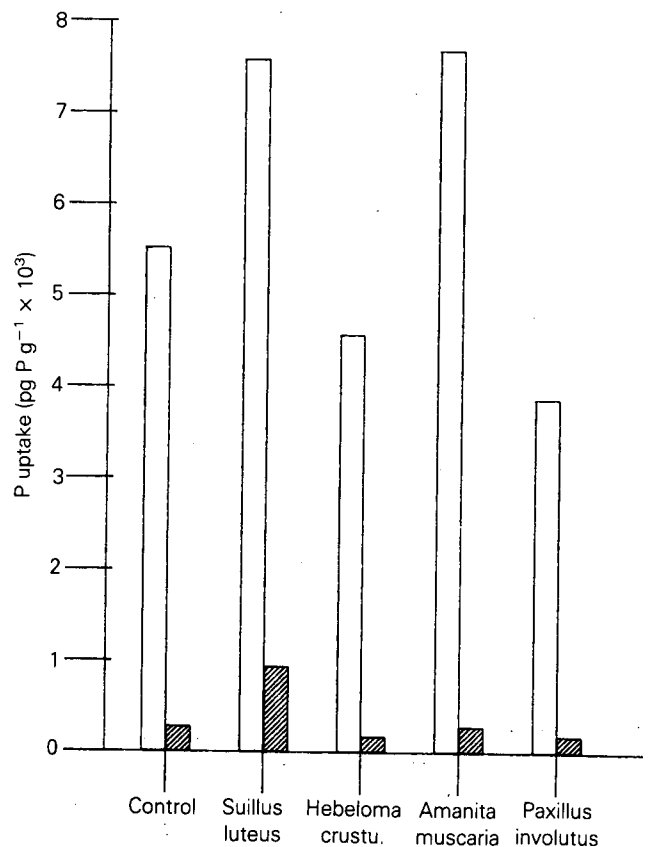


Figure 8. Uptake of phosphorus by lodgepole pine seedlings from peat inoculated with different mycorrhizas. Open bars represent shoots, hatched bars roots

Following afforestation, the availability of nutrients in the soil solution becomes increasingly dependent on the rate of breakdown of recalcitrant organic matter as the forest stand matures. Dighton and Mason (1985) put forward a diagrammatic model of such changes in their discussion of mycorrhizal dynamics in forest development (Figure 9). Where litter accumulation occurs, it would be advantageous for the tree if both stages of nutrient cycling (release of nutrients from organic matter by saprotrophic (decomposing) organisms and transfer into the tree roots via mycorrhizas) could be performed by the same fungal organism. This idea of 'direct cycling' of nutrients was first proposed

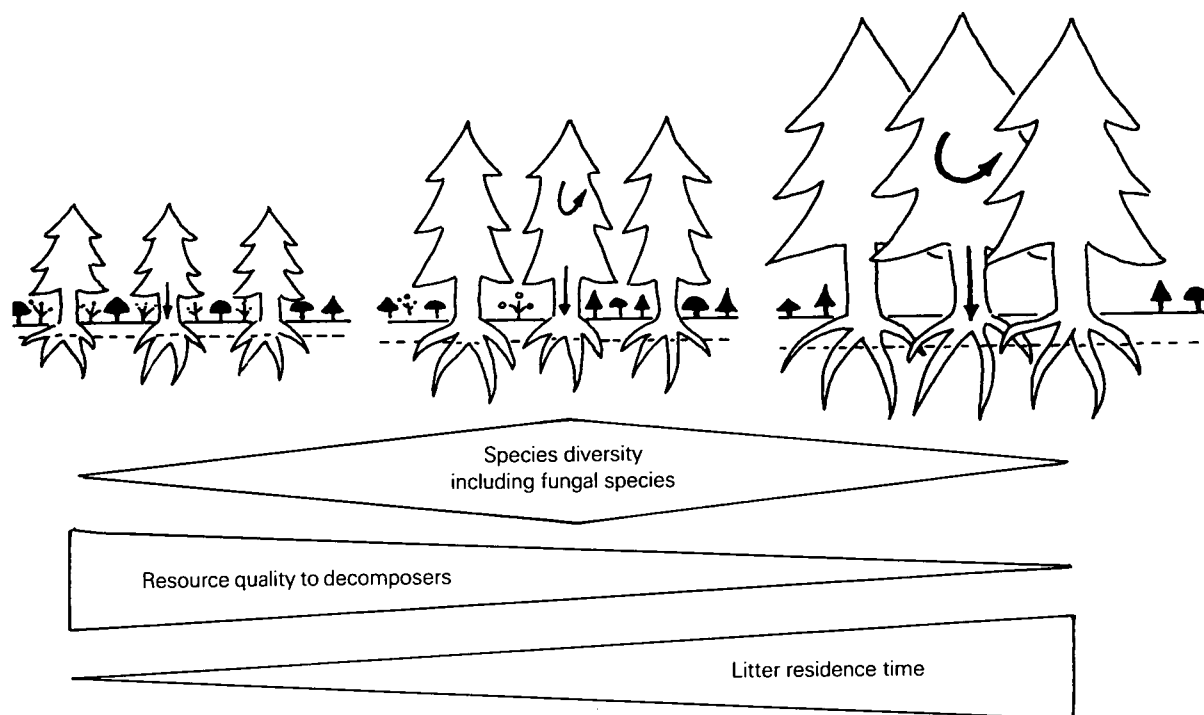


Figure 9. Diagrammatic representation of forest succession showing changes in ground flora and mycorrhizal fungi related to changes in resources entering the decomposer community (Dighton & Mason 1985)

by Went and Stark (1968), based on observations in tropical forests. There is evidence from our work and in the literature that certain mycorrhizal fungi, particularly under stressed environmental conditions, are able to act as decomposers. Dighton (1983) compared the phosphatase and phytase enzyme activity of known mycorrhizal and saprotrophic fungi, and found that some of the mycorrhizal species were capable of producing larger amounts of these enzymes than the saprotrophic fungi (Table 3). This finding implies a potential of these fungi to degrade complex organic phosphates. Similar studies have been made (Ho & Zak 1979; Alexander & Hardy 1981) to show phosphatase activity in other mycorrhizal fungi. Similarly, Giltrap (1982) has shown that polyphenol oxidases are produced by the mycorrhizal fungus *Lactarius* spp.; Norrans (1950), Oelbe (1982) and Linkins and Antibus (1981) have shown cellulase activity of *Tricholoma* and willow (*Salix* spp.) mycorrhizas.

For selection of potentially suitable mycorrhizal species/strains to enhance tree crop yield, a number of factors must be considered, many of which depend on

Table 3. Acid phosphatase production by fungal mycelia in Hagem's medium with 10 ppm orthophosphate-P or inositol hexaphosphate-P after 42 days' growth at 20 °C (Dighton 1983)

| Fungus                           | Phosphatase production<br>( $\mu\text{g phenol mg}^{-1}$ mycelium) |                             |
|----------------------------------|--|-----------------------------|
|                                  | Orthophosphate-P   | Inositol<br>hexaphosphate-P |
| <i>Hebeloma crustuliniforme</i>  | 29.8   | 23.9                        |
| <i>Lactarius rufus</i>           | 26.3   | 22.2                        |
| <i>Paxillus involutus</i>        | 17.1   | 10.1                        |
| <i>Lactarius pubescens</i>       | 12.8   | 7.6                         |
| <i>Amanita muscaria</i>          | 5.8  | 8.8                         |
| <i>Suillus luteus</i>            | 5.0  | 9.2                         |
| <i>Marasmius androsaceus</i> (s) | 20.0   | 1.7                         |
| <i>Mycena galopus</i> (s)        | 0.9  | 0.2                         |

(s) = saprotrophic fungus, the remainder are mycorrhizal

soil properties. From what stage of the succession should the fungi be selected? Have they been proven to enhance growth/nutrient content of the tree species under consideration? Are they able to compete against indigenous mycorrhizas or are they rapidly replaced?



Will the fungus survive in the soil type in which it is to be placed? If the soil is highly organic, containing recalcitrant litters, has the fungus the enzyme potential to act as a decomposer? What is the potential outcome of competition between the mycorrhizal fungus and indigenous saprotrophic fungi? Obviously, we are only just beginning to unravel some of the answers to these complex questions.

Nevertheless, present knowledge of the principles involved has now been applied to field trials near Hexham and near Jedburgh. Forest transplants were inoculated with selected mycorrhizal fungi prior to planting. Although the trials are only three to four years old, preliminary results for certain of the mycorrhizal selections look very promising, showing enhanced growth rates compared with the control transplants. The latter are likely to have mycorrhizas appropriate to a nutrient-rich mineral nursery soil, which is unlikely to be found in most field situations. It is now important for us to examine the potentially useful fungi to discover their physiological attributes which make them so successful.

### 13.4 Conclusion

Research into both tree mixtures and mycorrhizal inoculation is continuing at Merlewood and other research establishments. In the case of tree mixtures, the purpose is to understand fully the soil processes responsible for the success of this existing management practice, so that its full potential may be realized. In the case of mycorrhizal inoculation, the mechanisms by which it would operate have been defined from the outset, and research is directed towards developing a practical management technique. Both research areas have the common aim of enhancing woodland growth by manipulating nutrient cycles.

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