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Differences in the growth and ectomycorrhizal community of *Dryobalanops lanceolata* (Dipterocarpaceae) seedlings grown in ultramafic and non-ultramafic soils

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Abstract

Ultramafic soils have naturally high concentrations of metals and are often low in major plant nutrients. Plant species of non-ultramafic origin, such as *Dryobalanops lanceolata* (Dipterocarpaceae), generally grow less well on these soils. I found minimal changes in growth, but a 17% reduction in foliar potassium, when seedlings of *D. lanceolata* were grown in a non-native ultramafic soil when compared with a 'normal' tropical ultisol. There were, however, marked changes in the ectomycorrhizal community structure on the roots of *D. lanceolata*. *Cenococcum geophilum* was at least 10 times more common and *Inocybe* sp. was one and a half times more common in non-ultramafic soils, whereas Boletales sp. was over 30 times more common in the non-ultramafic soil. These changes may have been brought about by a number of edaphic differences between the two soil types, including high metal concentrations and differences in organic matter content.

Keywords: Borneo; Dipterocarps; *Dryobalanops*; Ectomycorrhizas; Potassium; Seedling growth; Serpentine; Soils; Tropical rain forest; Ultramafic

1. Introduction

Ultramafic soils are derived from igneous ultramafic rocks which have high concentrations of magnesium, iron and other metals such as nickel, chromium and cobalt. They have low silicon levels and are often lacking in the major plant nutrients: nitrogen, phosphorus and potassium. These distinctive edaphic factors provide unique challenges to the vegetation of these areas (Proctor 1999, 2003; Proctor and Nagy, 1992). A number of experiments have shown that, generally, plants of non-ultramafic origin grow less well in ultramafic soils or in solutions simulating ultramafic soils (Proctor, 1971; Johnston and Proctor, 1981; Nyberg Berglund et al., 2003).

Mycorrhizas play an essential role in the mineral nutrition of higher plants (Smith and Read, 1997, and references therein) and can also ameliorate the toxic effects of heavy metals (Leyval et al., 1997; Godbold et al., 1998; Jentschke and Godbold, 2000). These two complementary functions of mycorrhizas in the adaptation of plants to environmental stresses suggest that they may be exceptionally important in ultramafic soils. However, this assertion has been practically unexplored by plant ecologists and very few studies have been conducted to investigate the impact of ultramafic soils on ectomycorrhiza (EcM) communities (Iwamoto and Kitayama, 2002; Moser et al., 2005). There is a great diversity of mycorrhizal fungi and many fungal species can colonise an individual tree's roots (Bruns, 1995; Horton and Bruns, 2001); it has been suggested that a greater diversity of fungi is beneficial for the host plant due to a more efficient utilisation of resources (Leake, 2001).

Dipterocarps are the most important tree family in the lowland evergreen rain forests of Southeast Asia (Whitmore, 1984) and all, except one species, have been shown to possess EcMs (e.g., Singh, 1966; Nuhamara et al., 1985; Alexander and Högberg, 1986; Smits, 1992; Pampolina et al., 1995). *Dryobalanops lanceolata* Burck (Kapur paji) is

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a common dipterocarp species in the lowland evergreen rain forests of Sabah (a state of Malaysia on the island of Borneo). It is a shade-tolerant species which grows to a very large size on relatively more fertile soils and produces a medium hardwood which is commonly used for timber (Meijer and Wood, 1964). Dipterocarps are less common on ultramafic soils and *D. lanceolata* is absent from them (Meijer, 1964, J. Tangah, pers. comm.). This may be due to a different EcM community in these soils, which does not provide as many benefits to the seedlings as to those growing in non-ultramafic soils. The aim of this study is to determine whether it is possible for *D. lanceolata* to grow on ultramafic soils and to determine how the edaphic factors affect the growth and EcM community structure of this non-ultramafic species in an ultramafic soil.

2. Materials and methods

Seedlings of *D. lanceolata* (mean ± SE height 48.2 ± 1.9 cm, mean ± SE leaf number 8.0 ± 0.5) were grown in ultramafic soil collected from the Gunung Tawai Forest Reserve (5°33′N, 117°5′E) and non-ultramafic soil from the Kabili-Sepilok Forest Reserve (5°52′N, 117°56′E); both in Sabah. Soils were sieved to ca. 5 mm and any large stones or roots were removed. The seedlings were planted individually in plastic pots containing 1.2 l of the respective soil type in the nursery of the Sabah Forestry Department's Forest Research Centre in shade chambers, allowing transmission of 20% full sunlight ($<8.1\,mol\,m^{-2}\,day^{-1}$). Twelve seedlings were grown in each soil type and these were spread evenly across four shade chambers with three seedlings per chamber. They were watered by natural rainfall and were given supplemental water on days when there was no rain until the soil in the pots was saturated. Their positions were re-randomised monthly.

At the end of the experiment, after 6 months, leaf length and width were measured and the leaf area was calculated using the equation of Bungard et al. (2002):

$$\text{Leaf area}(cm^2) = -0.28 + 0.007 \times (\text{length}\,(mm) \times \text{width}\,(mm)).$$

Seedlings were harvested, divided into four fractions (leaves, stem and branches, tap root, and fine roots) and dried at 50 °C for at least 1 week. Biomass allocation patterns were calculated using the equation

$$\text{Mass fraction} = \text{biomass of fraction}/\text{total biomass of seedling}.$$

For the determination of foliar nutrient concentrations, plant tissues were digested in a salicylic/sulphuric acid mix ($33\,g\,l^{-1}$) with a lithium sulphate/copper sulphate (10:1 ratio) catalyst. Phosphorus and nitrogen were analysed on an auto-analyser (Tecator 5042 Detector and 5012 Analyser, Foss UK Ltd., Didcot, UK) using the ammonium molybdate–stannous chloride method (Tecator Ltd, 1983) and gas diffusion method (Tecator, 1984), respectively. Potassium, Ca and Mg were analysed by atomic absorption spectrophotometry (Perkin-Elmer 2100 Atomic Absorption Spectrophotometer, Beaconsfield, UK). Around 200 root tips from each seedling were examined under a dissecting microscope (Olympus SZH, Olympus Optical Co. Ltd., Tokyo, Japan) to determine individual EcM morphotypes. The total percentage EcM colonisation and the Shannon–Wiener diversity index of EcM morphotypes were calculated as in Brearley et al. (2003). All factors examined were compared between the two soil types using *t*-tests ($\log_e$ transformations were carried out where necessary), with the exception of colonisation by the individual EcM morphotypes where Mann–Whitney tests were used. All statistical analyses were carried out using Minitab 13.31 (Minitab Inc., State College, PA, USA).

3. Results

Ultramafic soils from Gunung Tawai were less acidic, had more organic matter, a higher Ni concentration and a higher Mg:Ca ratio than non-ultramafic soils from Kabili-Sepilok (Table 1). Full details of soil sampling and chemical analyses are given in Brearley (2005) for Gunung Tawai and Brearley (2003; unpublished Ph.D. Thesis, University of Sheffield, UK) for Kabili-Sepilok, but due to different methods and equipment used for some of the analyses, formal statistical comparisons are not valid.

All 12 seedlings grown in the ultramafic soil survived to the end of the experiment, whereas 10 of the seedlings grown in the non-ultramafic soil survived. There were no differences in the biomass or leaf area of *D. lanceolata* grown in the two soil types (Table 2), indicating that the growth of this non-ultramafic species was not inhibited when grown in an ultramafic soil. Foliar nutrient concentrations were not different between seedlings grown in the two soil types, with the exception of potassium which was 17% higher in seedlings grown in the non-ultramafic soil (Table 2). Whilst there was no effect of the soil type on total percentage EcM colonisation, there was a marked effect on the EcM community structure (Table 3). *Cenococcum geophilum* was at least 10 times more common

Table 1
Comparison of tropical ultramafic soil from Gunung Tawai Forest Reserve, Sabah, with non-ultramafic soil from Kabili-Sepilok Forest Reserve, Sabah

	Ultramafic (Gunung Tawai)	Non-ultramafic (Kabili-Sepilok)
pH (H_2O)	5.3 ± 0.06	4.6 ± 0.05
Loss on ignition (%)	12.6 ± 0.30	3.7 ± 0.22
N total (%)	0.18 ± 0.01	0.13 ± 0.01
P total ($\mu g\,g^{-1}$)	201 ± 9.4	232 ± 6.9
K^+ exch. ($meq\,100\,g^{-1}$)	0.17 ± 0.01	0.29 ± 0.02
Mg^{2+} exch. ($meq\,100\,g^{-1}$)	1.38 ± 0.04	2.19 ± 0.24
Ca^{2+} exch. ($meq\,100\,g^{-1}$)	0.86 ± 0.04	2.12 ± 0.45
Ni extr. ($\mu g\,g^{-1}$)	10.80 ± 0.36	2.00 ± 0.71

All values are mean ± SE.

Table 2
Biomass, leaf area, biomass allocation and foliar nutrient concentrations of *Dryobalanops lanceolata* following growth for six months in tropical ultramafic soil (from Gunung Tawai Forest Reserve, Sabah) or non-ultramafic soil (from Kabili-Sepilok Forest Reserve, Sabah)

	Ultramafic	Non-ultramafic	*P*
Biomass (g)	18.6±1.3	15.5±1.1	0.087
Leaf area (cm^2)	626±71.3	457±58.4	0.089
Leaf mass fraction	0.22±0.02	0.21±0.02	0.86
Stem mass fraction	0.48±0.02	0.45±0.02	0.36
Root mass fraction[a]	0.30±0.01	0.33±0.01	0.061
Fine root mass fraction[b]	0.089±0.01	0.098±0.01	0.55
N ($mg\,g^{-1}$)[b]	11.66±1.33	9.72±0.75	0.23
P ($mg\,g^{-1}$)[b]	0.84±0.080	0.90±0.064	0.44
K ($mg\,g^{-1}$)	9.45±0.48	11.08±0.42	0.021
Ca ($mg\,g^{-1}$)	2.34±0.16	2.73±0.44	0.42
Mg ($mg\,g^{-1}$)	1.95±0.15	1.77±0.13	0.40

All values are mean±SE.
[a]Includes fine root biomass.
[b]Log_e transformation before analysis.

Table 3
Percentage colonisation of *D. lanceolata* by six EcM morphotypes following growth for 6 months in tropical ultramafic soil (from Gunung Tawai Forest Reserve, Sabah) or non-ultramafic soil (from Kabili-Sepilok Forest Reserve, Sabah). The Shannon–Wiener diversity index is also shown

	Ultramafic	Non-ultramafic	*P*
Percentage EcM colonisation	70.1±5.8	66.5±3.9	0.63
Basidiomycete sp.	0.0 (0.0 & 0.0)	0.0 (0.0 & 0.0)	ND[a]
Boletales sp.	35.8 (31.3 & 44.2)	0.0 (0.0 & 0.9)	0.004
Cenococcum geophilum Fr.	0.0 (0.0 & 0.4)	5.2 (0.6 & 14.7)	0.010
Inocybe sp.	33.8 (19.6 & 36.5)	47.7 (45.0 & 65.8)	0.003
Riessiella sp.	0.0 (0.0 & 0.0)	0.0 (0.0 & 0.0)	ND[a]
Thelephorales sp.	0.0 (0.0 & 6.0)	0.0 (0.0 & 0.0)	0.38
Shannon–Wiener diversity index	0.72±0.05	0.51±0.06	0.020

Values for percentage EcM colonisation and Shannon–Wiener diversity index are mean±SE; values for the colonisation by individual morphotypes are median with lower & upper quartile in parentheses.
[a]Could not be determined due to all values being zero in one of the soil types.

and *Inocybe* sp. was around 1.5 times more common in the non-ultramafic soil than in the ultramafic soil. The opposite pattern was seen for the Boletales sp., which was over 30 times more common in the ultramafic soil than in the non-ultramafic soil (Table 3).

4. Discussion

This study has shown that *D. lanceolata* grows equally well in its non-native ultramafic soil as in 'normal' non-ultramafic soil. This was somewhat unexpected, but may be because the soil used in this experiment did not have as high metal concentrations as some other ultramafic soils (see Roberts and Proctor, 1992, for examples). I have previously shown that when calcium was added to seedlings of *D. lanceolata* grown in the same ultramafic soil, there was no effect on growth, indicating that magnesium toxicity was not an important factor preventing growth in this soil (Brearley, 2005). The only difference in foliar nutrient concentrations in this experiment was the lower potassium concentration in the seedlings grown in the ultramafic soil. This lends support to the hypothesis of potassium limitation preventing seedlings of *D. lanceolata* gaining dominance on these soils (Brearley, 2005). Other than nutrient limitation, it may be fruitful to look for biotic factors that may prevent *D. lanceolata* from gaining dominance on ultramafic soils, such as insect herbivory or fungal pathogen attack.

The effect of different soil types on the EcM community on the roots of *D. lanceolata* was much more marked than the effects on seedling growth. There may be a number of reasons for these changes in the EcM community structure, including changes in soil pH, nutrient concentrations, organic matter and moisture content. The most important factor is probably the difference in metal concentrations between the two soils, with the ultramafic soil having greater concentrations of magnesium and nickel as well as other, unmeasured, metals. It is likely that the greater concentrations of metals negatively affected C. *geophilum* and *Inocybe* sp. However, I have previously suggested that *C. geophilum* is more common and/or has a competitive advantage in soils that are more likely to dry out (Brearley et al., 2003). In the current study, non-ultramafic soils are considered more likely to dry out as they have a lower proportion of organic matter. The Boletales sp. was the only morphotype which was more common in the ultramafic soil, suggesting some tolerance to higher metal concentrations. Interestingly, Markkola et al. (2002) found nickel deposition to have an effect on only one EcM morphotype of the nine that they described from the roots of *Pinus sylvestris* L., indicating that factors other than soil nickel concentrations may be more important in structuring EcM communities. The next logical step would be to carry out experiments *in vitro* or with single isolates on the roots of host seedlings, examining the responses of these EcM species to various perturbations such as heavy metals, nutrients and water stress. The much lower colonisation by *C. geophilum* and *Inocybe* sp., and greater colonisation by Boletales sp., might be leading to *D. lanceolata* being absent from ultramafic soils. Whilst seedling growth was not different between the two soils types in the nursery, these EcM species might affect seedling performance in the field through their effects on nutrient transfer or perhaps by affecting levels of insect herbivory. Alternatively, they might play a greater role in nutrient uptake at a later life stage not examined during the course of this experiment.

Diversity of EcM types per seedling was greater in the ultramafic soils. This concurs with results obtained by Iwamoto and Kitayama (2002), who compared EcM colonisation on roots from ultramafic and sedimentary

soils from Mount Kinabalu, Sabah. They also found a greater number of EcM morphotypes per soil core from ultramafic soils, and a greater total number of EcM morphotypes were recovered from ultramafic soil (20) than from sedimentary soil (7). This compares with data from Moser et al. (2005), who found no differences in EcM diversity in soil cores collected from ultramafic and non-ultramafic sites in Oregon, USA. Out of 74 EcM morphotypes they examined, 46 were found in the ultramafic sites and 42 were found in the non-ultramafic sites.

To conclude, this study has shown that *D. lanceolata* is not excluded from its non-native ultramafic soils due to edaphic factors as it grows equally well there in the seedling stage. However, the EcM community on the roots of *D. lanceolata* was markedly affected in its non-native soils, which may lead to changes in performance at a later life stage and ultimately prevent successful growth of *D. lanceolata* in ultramafic soils.

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