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# Arbuscular Mycorrhizal Status of Indigenous Tree Species Used to Restore Seasonally Dry Tropical Forest in Northern Thailand

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**Abstract:** Arbuscular Mycorrhizal (AM) status of native plants in the tropical forest of northern Thailand was surveyed. Twenty four framework tree species, used to forest restoration were examined at 3 sites: FORRU's research tree Nursery (FN), Forest Restoration plot (FR) and Natural Forest (NF). Eleven dominant herb species were examined at 2 sites: Degraded Watershed (DW) and Forest Soil extraction area (FS). Rhizosphere soil samples were collected and AM fungal spores were counted and identified morphologically. Most plant species were intensively colonized by AM fungi except *Cyperus cyperoides*. Twenty four AM species were identified: *Glomus* (15 species), *Acaulospora* (6 species) and *Scutellospora* (3 species). *Glomus rubiforme* was the dominant species. Spore density varied from 16.1 to 97.4 per 100 g soil (averaged 59.7). Spore number at DW and FS were 129 and 479 spores, respectively, with species richness of 6 and 8, respectively. Spore number at FN, FR and NF were 1,152, 2,337 and 1,376 spores, respectively, with species richness of 17, 21 and 15, respectively. The AM diversity was lower in the sites dominated by herbs than in sites examined for trees. In the deforested sites, reduced plant diversity was related with reduced mycorrhizal diversity. In contrast, the trial plot had the highest AM fungal community. Therefore, the forest restoration techniques allow tree species grown in nursery to become AM associated. The association is still maintained after planting out trees in restored area.

**Key words:** Arbuscular mycorrhizal fungi, framework tree species, herb species, tropical forest restoration

## INTRODUCTION

Tropical deforestation causes forest fragmentation and permits the extensive areas of degraded land. This leads to losses of biodiversity, a decline in soil fertility and deterioration of soil physical and biological properties. The seasonally dry tropical forests of Doi Suthep-Pui National Park in northern Thailand support more than 480 indigenous tree species. Deforestation within the park has had adverse consequences for biodiversity and environmental quality. Previous reforestation programs, to counteract this problem, used mainly exotic tree species. This was partly due to lack of knowledge about the environmental requirements of indigenous trees. One method of forest restoration that have proved very successful in Queensland, Australia, is the so-called framework species method (Goosem and Tucker, 1995; FORRU, 2006), which involves planting mixtures of several indigenous tree species in a single step. Therefore, the Forest Restoration Research Unit (FORRU) at Chiang Mai University (CMU) began assessing the suitability of a wide range of indigenous trees to restore evergreen forest

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on degraded sites within the national park. The unit looked for trees likely to act as framework species i.e., those that would out-compete weeds and attract seed-dispersing animals. Candidate framework species were selected, grown in a nursery and tested in trial plot, established in 1998 in a degraded watershed area in the north of national park (Elliott *et al*., 2003).

Most sites requiring forest restoration have soil with low-fertility and a low inoculum potential of microorganisms beneficial to plants, such as Arbuscular Mycorrhizal (AM) fungi (Setiadi, 2000; Korb *et al*., 2003). AM fungi are only one of numerable microorganisms in soil that provide a direct link between plant roots and the soil matrix. AM fungi are widespread in natural ecosystems; play a crucial role in the mineral nutrition of forest trees and provide important nutrient-acquiring mechanisms (Koide and Mosse, 2004). Inoculation with effective AM fungi, combined with early intense colonization of seedlings in tree nurseries, stimulates plant growth and establishment in the field under nutrient-deficient and drought-stressed conditions (Setiadi, 2000). Thus, the capacity of potential framework species to associate with indigenous AM fungi is a very important strategy for forest restoration. The purposes of the present study were to: (1) obtain information on the AM status of 24 framework trees and 11 weedy herbs in Doi Suthep-Pui National Park (2) assess the diversity and distribution of AM fungi associated with plant species in natural forest, degraded area and restoration area. In addition this study was carried out to select AM fungi to produce inoculum for improving nursery tree seedling performance and promoting sapling growth in reforestation areas.

## MATERIALS AND METHODS

### Study Sites

This study was carried out at 5 sites within Doi Suthep-Pui National Park, Chiang Mai, Thailand. There are two kinds of forest, including deciduous forest (from the lowlands up to about 950 m a.s.l) and Evergreen Forest (EGF) (from about 950 m a.s.l to the summit, 1,685 m a.s.l). Average annual rainfall was 2094.9 mm. Temperatures range from 4.5°C in December to 35.5°C in March (Elliott *et al*., 2003).

FORRU's research tree Nursery (FN) is situated near the accommodation center of national Park (18°50′N, 98°50′E) at about 1,000 m a.s.l. FORRU screened indigenous tree species to select potential framework species for field trials. Saplings were grown in black plastic bags containing a nursery potting medium of primary evergreen forest soil, coconut husk and peanut husk (2:1:1).

Forest Restoration plot (FR) was established in a degraded watershed area (18°52′N, 98°51′E). Trial plot was positioned along the ridges of a degraded watershed area at 1,207-1,310 m a.s.l. At least 48 nursery saplings of each of 24 framework species were planted out randomly in the field plot.

The Natural evergreen Forest (NF) of Doi Suthep-Pui is one of the most luxuriant in northern Thailand. The forest supports many indigenous tree species, but the framework species in this sampling are sparsely scattered across the national park at 1,050-1,475 m a.s.l.

The Degraded Watershed area (DW) had originally been covered in EGF, but the forest was cleared for cultivation about 20 years previously. Degraded areas at about 1,207-1,310 m a.s.l, near the field plot still supported a few remnant forest trees and were dominated by herbaceous weeds.

The Forest Soil extraction area (FS) was in disturbed EGF at 1,685 m a.s.l. The area, surrounded by natural forest was covered with a mature tree canopy and herbaceous weeds. The forest soil is rich in organic matter with high moisture-holding capacity.

### Study Plants

Among the plants of the evergreen forest zone of national park, 24 potential framework species (representing 19 families) were selected for study. All tree species are reported to be multipurpose and

suitable for acceleration forest regeneration. Root and soil samples from saplings at the FN, planted trees at the FR and mature trees at the NF of each species were collected. At the other study sites, dominant herb species (representing 5 families) were chosen as study plants; 8 species were sampled at the DW and 6 species at the FS.

**Root and Soil Sampling**

Sampling of plant roots and their rhizosphere soil took place at 5 study sites between February and April 2005 (dry season). Soil and root samples (about 500 g) were collected to a depth of 10 cm of three individual plants of each species and stored at 4°C until analyzed.

**Estimation of Mycorrhizal Colonization**

Roots of study plants were separated from each soil sample. The root samples were cleared in 10% (w/v) KOH at 121°C for 15 min and rinsed with water on a 90 μm sieve. Cleared roots were stained with 0.5% acid fuchsin (Brundrett *et al*., 1996). Thirty stained root segments from each plant (about 1 cm long) were taken at random and mounted on microscopic slides to assess mycorrhizal colonization (McGonigle *et al*., 1990).

**Spore Extraction and Counting**

Spores were isolated from 100 g air-dried soil taken from each field soil sample using the wet-sieving method as described by An *et al*. (1990). Spores were recovered by filtering through a 53 μm sieve onto filter paper. The intact spores on filter paper were counted under a dissecting microscope. A sporocarp was counted as one unit.

**Identification of AM Fungi**

Spores of AM fungi isolated from the field soils were mounted on glass slides in polyvinyl lactic acid (PVA) or PVA+Melzers reagent (Morton, 1988). The spores were identified according to morphological characteristics of original published species descriptions and using the Internet information from the INVAM website (http://invam.caf.wvu.edu).

**Data Analysis**

Species richness, spore density, spore number, frequency and relative abundance of AM fungi were expressed as follows: species richness = species observed per sample; spore density = No. of spores per 100 g dried field soil; spore number = No. of spores observed per total samples; frequency = (No. of the samples in which the species or genus observed per total samples) × 100%; relative abundance = (No. of spores of a species or a genus per total spores) × 100%. The data were subjected to one-way ANOVA. Duncan·s Multiple Range Test ($p<0.05$) was used to compare means.

## RESULTS

**Arbuscular Mycorrhizal Status of Plant Species and Extent of Mycorrhizal Colonization**

All plants studied formed AM symbioses. The extent of AM colonization (Table 1 and 2) was uneven among the different growth stages of individual tree species (saplings and mature) and ground herbs. All the typical AM features, such as arbuscules, vesicles, intracellular hyphal coils, extra and intraradical hyphae, were observed in the samples. Most plant species were usually densely colonized by intraradical hyphae, followed by arbuscules and vesicles in the root cortical tissues. Colonization percentages in tree roots across all sites, ranged from 56.0% for *Ficus glaberrima* to 98.3% for *Macaranga denticulata*. Seven species had colonization percentages higher than 90%: *M. denticulata*,

Table 1: Mean arbuscular mycorrhizal colonization percentages±S.E. (per 30 root pieces of plant species) (n = 2) and spore density±SE (per 100 g dry wt soil) (n = 3) associated with different herb species at two study sites

| | Study site | | | |
|---|---|---|---|---|
| | Degraded watershed area | | Forest soil extraction area | |
| Herb species | Colonization (%) | Spore density | Colonization (%) | Spore density |
| *Ageratum conyzoides* L. (Compositae) | 95.10±0.36ns | 12.67±1.20b | 81.71±8.03ns | 84.66±14.33a |
| *Anaphalis margaritacea* (L.) Bth. and Hk.f. (Compositae) | 65.80±12.97ns | 7.67±1.20b | 99.09±0.91ns | 52.67±6.06a |
| *Conyza sumatrensis* (Retz.) Walk. (Compositae) | 47.76±9.50 | 8.00±1.53 | | |
| *Crassocephalum crepidioides* (Bth.) S. Moore (Compositae) | 83.22±7.52 | 15.67±2.60 | | |
| *Cyperus cyperoides* (L.) O.K. (Cyperaceae) | 2.44±2.44 | 32.67±2.40 | | |
| *Eupatorium adenophorum* Spreng. (Compositae) | | | 84.01±1.84 | 64.33±8.51 |
| *Microstegium vagans*(Nees ex Steud.) A. Camus (Gramineae) | | | 73.46±6.80 | 112.67±11.46 |
| *Mitracarpus villosus* (SW.) DC. (Rubiaceae) | 81.31±8.41 | 28.00±6.08 | | |
| *Pteridium aquilinum* (L.) Kuhn ssp. (Dennstaedtiaceae) | 82.26±0.16b | 7.67±1.86b | 100.00±0.00a | 36.33±6.12a |
| *Spilanthes paniculata* Wall. ex DC. (Compositae) | | | 91.58±8.42 | 127.67±20.10 |
| *Thysanolaena latifolia* (Roxb. ex Horn.) Honda (Gramineae) | 89.84±4.94 | 16.67±3.71 | | |
| Average spore density[a] | | 16.13±3.37d | | 79.72±14.44b |
| Spore number | | 129.00 | | 479.00 |

Letter(s) indicate significant differences within each row at $p<0.05$ as determined by ANOVA and Duncan's Multiple Range Test; ns, not significant, [a]: Values were analyzed to compare statistically across all five study sites in Table 1 and 2

*Nyssa javanica*, *Melia toosendan*, *Hovenia dulcis*, *Heynea trijuga*, *Erythrina subumbrans* and *Rhus rhetsoides*. *Cyperus cyperoides*, which is considered to be non-mycorrhizal or rarely forming mycorrhizas, was also found to be colonized by AM fungi in this study, but had a low (<2.4%) colonization percentage.

**Arbuscular Mycorrhizal Species and Frequency of Occurrence**

Five thousand four hundred and seventy three AM fungal spores (including sporocarps) were retrieved from the 86 composite soil samples from all 5 study sites, representing 24 AM species, identified according to published descriptions. The frequency (F%) and relative abundance (RA%) of the genera and species of AM fungi are presented in Table 3. Most of the isolated species belonged to the family *Glomaceae*, all of which were *Glomus* (15 species). The most abundant species present was *G. rubiforme*. Four species had formerly been assigned to *Sclerocystis* (*G. clavisporum*, *G. coremioides*, *G. rubiforme* and *G. sinuosum*). Six species were in the family *Acaulosporaceae*, all of which were in the genus *Acaulospora*. The most abundant species present was *A. scrobiculata*. Three species were members of the family *Gigasporaceae* and belonged to the genus *Scutellospora*. Species in the genera *Archaeospora*, *Paraglomus*, *Entrophospora* and *Gigaspora* were not found.

Some species of AM fungi appeared to be generalists since they were found in the rhizospheres of study plant species at virtually all study sites: *A. elegans*, *A. scrobiculata*, *G. ambisporum*, *G. microcarpum*, *G. rubiforme* and *G. sinuosum*. Of these, *A. scrobiculata*, *G. microcarpum* and *G. rubiforme* were dominant at the FS, FN, RF and NF, but they were also abundant at the DW. Some species (*G. microaggregatum* and *S. heterogama*) were found in the soils of disturbed areas both at the DW and FR, but were absent from natural evergreen forest soils at the FS, FN and NF. Some AM species (*A. bireticulata* and *S. pellucida*) were found in the soils of the FN and FR but were absent from the soils at the DW, FS and NF. In the rhizosphere of herb species, some species (*G. microaggregatum*, *G. sinuosum* and *S. heterogama*) were found in the DW but were absent from the FS.

Table 2: Mean arbuscular mycorrhizal colonization percentages ±SE (per 30 root pieces of plant species) (n = 2) and spore density ±SE (per 100 g dry wt. soil) (n = 3) associated with different tree species at three study sites

| | Study site | | | | | |
|---|---|---|---|---|---|---|
| | FORRU's research nursery | | Forest restoration plots | | Natural evergreen forests | |
| Tree species | Colonization (%) | Spore density | Colonization (%) | Spore density | Colonization (%) | Spore density |
| *Acrocarpus fraxinifolius* Wight ex Arn. (Caesalpinioideae) | 65.56±4.44b | 120.67±4.33a | 94.77±0.83a | 60.67±6.36b | 74.38±4.20b | 12.00±1.73c |
| *Balakata baccata* (Roxb.) Ess. (Euphorbiaceae) | 97.22±2.78a | 4.00±2.08b | 100.00±0.00a | 216.67±32.69a | 26.14±1.14b | 8.67±2.33b |
| *Castanopsis acuminatissima* (Bl.) A. DC. (Fagaceae) | 11.46±1.16b | 4.33±2.19b | 83.42±14.20a | 16.00±2.08a | 82.12±0.64a | 8.00±2.65ab |
| *Erythrina subumbrans* (Hassk.) Merr. (Papilionoideae) | 83.22±9.48ns | 8.33±1.45b | 98.34±1.66ns | 75.67±4.63a | 94.54±5.46ns | 4.33±2.33b |
| *Ficus altissima* Bl. (Moraceae) | 93.28±2.96a | 32.67±5.17b | 94.76±5.24a | 59.67±8.29a | 51.94±5.94b | 4.33±0.88c |
| *Ficus benjamina* L. var. *benjamina* (Moraceae) | 75.66±19.34ns | 32.67±2.85b | 75.18±2.90ns | 23.67±2.96b | 87.05±1.63ns | 119.67±6.33a |
| *Ficus glaberrima* Bl. var. *glaberrima* (Moraceae) | 69.64±17.46a | 16.67±3.18a | 89.68±3.18a | 4.00±2.31b | 8.61±3.25b | 8.33±2.03ab |
| *Ficus hispida* L. f. var. *hispida* (Moraceae) | 70.92±4.64ns | 29.00±2.08b | 49.98±0.86ns | 60.00±7.81b | 55.99±8.92ns | 251.33±34.12a |
| *Ficus racemosa* L. var. *racemosa* (Moraceae) | 88.14±9.92a | 68.67±12.35b | 98.41±1.59a | 204.33±22.82a | 32.68±7.68b | 4.00±1.00c |
| *Ficus subulata* Bl. var. *subulata* (Moraceae) | 39.75±4.37b | 39.67±3.48b | 69.68±20.32ab | 56.67±3.38b | 98.86±1.14a | 124.66±20.50a |
| *Glochidion kerrii* Craib (Euphorbiaceae) | 83.35±3.66a | 36.67±1.76b | 89.06±0.17a | 23.67±3.53b | 64.00±4.62b | 64.67±6.94a |
| *Gmelina arborea* Roxb. (Verbenaceae) | 98.00±2.00ns | 32.33±7.88ab | 100.00±0.00ns | 48.33±4.91a | 67.30±14.52ns | 23.67±5.24b |
| *Heynea trijuga* Roxb. ex Sims (Meliaceae) | 84.48±7.38ns | 19.67±4.33b | 99.28±0.72ns | 595.67±58.89a | 94.09±0.65ns | 8.33±1.76b |
| *Hovenia dulcis* Thunb. (Rhamnaceae) | 100.00±0.00a | 28.00±2.00c | 84.35±2.53b | 292.00±12.17a | 93.73±4.60ab | 64.67±6.89b |
| *Macaranga denticulata* (Bl.) M.-A. (Euphorbiaceae) | 100.00±0.00ns | 56.67±13.30b | 96.22±2.28ns | 128.33±2.33a | 98.68±1.32ns | 64.00±19.55b |
| *Machilus bombycina* King ex Hk.f. (Lauraceae) | 75.43±2.35ns | 51.67±2.90a | 49.45±25.06ns | 55.33±9.24a | 95.34±0.90ns | 15.67±6.69b |
| *Melia toosendan* Sieb. and Zuc. (Meliaceae) | 92.08±6.38ns | 20.33±2.96b | 96.94±3.06ns | 104.33±21.49a | 93.02±5.30ns | 8.00±2.65b |
| *Michelia baillonii* Pierre (Magnoliaceae) | 100.00±0.00a | 40.00±5.03a | 97.66±2.34a | 15.67±0.33b | 68.61±6.39b | 24.00±6.03b |
| *Nyssa javanica* (Bl.) Wang. (Nyssaceae) | 92.19±7.81ns | 108.33±4.63b | 100.00±0.00ns | 172.00±22.37ab | 98.18±1.82ns | 200.67±23.69a |
| *Prunus cerasoides* D. Don (Rosaceae) | 76.85±6.48a | 32.00±12.06b | 21.24±5.68b | 43.33±2.73b | 87.72±7.72a | 112.67±9.26a |
| *Rhus rhetsoides* Craib (Anacardiaceae) | 100.00±0.00ns | 204.33±12.84a | 91.26±7.26ns | 40.33±3.28b | 84.74±1.80ns | 28.00±4.58b |
| *Sapindus rarak* DC. (Sapindaceae) | 88.87±2.20a | 28.67±2.60b | 82.10±0.16ab | 12.33±2.40b | 62.67±9.55b | 196.33±25.64a |
| *Sarcosperma arboreum* Bth. (Sapotaceae) | 40.90±23.25ns | 8.67±2.91b | 92.38±0.48ns | 24.33±0.88a | 55.67±8.61ns | 12.33±3.71b |
| *Spondias axillaris* Roxb. (Anacardiaceae) | 93.34±3.48ns | 128.33±17.84a | 72.20±13.50ns | 4.00±1.53b | 51.24±9.94ns | 8.00±0.58b |
| Average spore density* | | 48.01±9.72c | | 97.38±26.55a | | 57.35±14.80c |
| Spore number | | 1152.00 | | 2337.00 | | 1376.00 |

Letter(s) indicate significant differences within each row at $p<0.05$ as determined by ANOVA and Duncan's Multiple Range Test; ns: Not significant, *: Values were analyzed to compare statistically across all five study sites in Table 1 and 2

Fourteen of the 15 AM species recorded in natural evergreen forest were maintained in the forest restoration plot (all except *G. fulvum*). Furthermore, the restoration plot supported 7 additional recruit species that were not recorded in the rhizospheres of forest trees (Table 3). Even in the nursery, only 4 of the AM species recorded in natural evergreen forest were absent. It should be noted that forest soil, incorporated into the nursery potting medium carried only 5 of the 15 forest AM species. Therefore, at least 9 species must have colonized the nursery potting medium as the trees were growing in their containers on the ground.

**Spore Abundance and Species Richness of Arbuscular Mycorrhizal Fungi**

From all composite soil samples, spore density and species richness of AM fungi differed substantially. Average spore densities in the soils at 5 study sites ranged from 16.1 to 97.4 spores per

Table 3: Frequency (F%) and relative abundance (RA%) of genera and species of arbuscular mycorrhizal fungi in the rhizosphere of different plant species for each study site

| | Study site | | | |
|---|---|---|---|---|
| | Degraded watershed area | | Forest soil extraction area | |
| AM fungal species | F% | RA% | F% | RA% |
| ***Acaulospora*** | 75.00 | 52.71 | 66.67 | 29.48 |
| 1. *A. bireticulata* Rothwell and Trappe | | | | |
| 2. *A. elegans* Trappe and Gerd. | | | 33.33 | 9.27 |
| 3. *A. foveata* Trappe and Janos | | | | |
| 4. *A. laevis* Gerd. and Trappe | | | | |
| 5. *A. mellea* Spain and Schenck | | | 33.33 | 5.02 |
| 6. *A. scrobiculata* Trappe | 75.00 | 52.71 | 50.00 | 15.19 |
| ***Glomus*** | 87.50 | 44.19 | 100.00 | 68.78 |
| 7. *G. aggregatum* Schenck and Smith | | | | |
| 8. *G. ambisporum* Smith and Schenck | | | 33.33 | 12.54 |
| 9. *G. clavisporum* Trappe | | | | |
| 10. *G. coremioides* Berk. and Broome | | | | |
| 11. *G. fulvum* (Berke. and Broome) Trappe and Gerd. | | | | |
| 12. *G. intraradices* Schenck and Smith | | | | |
| 13. *G. microaggregatum* Koske, Gemma and Olexia | 12.50 | 3.62 | | |
| 14. *G. microcarpum* Iqbal and Bushra | 25.00 | 6.71 | 33.33 | 22.58 |
| 15. *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe | | | | |
| 16. *G. multicaule* Gerd. and Bakshi | | | | |
| 17. *G. rubiforme* Gerd. and Trappe | 50.00 | 25.07 | 100.00 | 31.22 |
| 18. *G. scintillans* Rose and Trappe | | | | |
| 19. *G. sinuosum* Gerd. and Bakshi | 25.00 | 8.79 | | |
| 20. *G. tortuosum* Schenck and Smith | | | | |
| 21. *G. viscosum* Nicol. | | | 16.67 | 2.44 |
| ***Scutellospora*** | 12.50 | 3.10 | 16.67 | 1.74 |
| 22. *S. gregaria* (Schenck and Nicol.) Walker and Sanders | | | | |
| 23. *S. heterogama* Walker and Sanders | 12.50 | 3.10 | | |
| 24. *S. pellucida* (Nicol. and Schenck) Walker and Sanders | | | 16.67 | 1.74 |
| Species richness | 6.00 | | 8.00 | |

| | Study site | | | | | |
|---|---|---|---|---|---|---|
| | FORRU's research nursery | | Forest restoration plots | | Natural evergreen forests | |
| AM fungal species | F% | RA% | F% | RA% | F% | RA% |
| ***Acaulospora*** | 66.67 | 40.50 | 66.67 | 43.63 | 33.33 | 5.91 |
| 1. *A. bireticulata* Rothwell and Trappe | 4.17 | 0.66 | 4.17 | 0.33 | | |
| 2. *A. elegans* Trappe and Gerd. | 16.67 | 2.37 | 50.00 | 39.17 | 16.67 | 4.38 |
| 3. *A. foveata* Trappe and Janos | 4.17 | 0.69 | 8.33 | 1.36 | 4.17 | 0.31 |
| 4. *A. laevis* Gerd. and Trappe | 4.17 | 0.40 | 4.17 | 0.19 | 4.17 | 0.31 |
| 5. *A. mellea* Spain and Schenck | 20.83 | 6.31 | 4.17 | 0.36 | | |
| 6. *A. scrobiculata* Trappe | 58.33 | 30.06 | 20.83 | 2.24 | 12.50 | 0.90 |
| ***Glomus*** | 91.67 | 56.03 | 87.50 | 49.32 | 91.67 | 94.09 |
| 7. *G. aggregatum* Schenck and Smith | | | 8.33 | 0.38 | 4.17 | 0.29 |
| 8. *G. ambisporum* Smith and Schenck | 54.17 | 14.23 | 12.50 | 3.05 | 58.33 | 31.19 |
| 9. *G. clavisporum* Trappe | | | 8.33 | 0.33 | 4.17 | 0.61 |
| 10. *G. coremioides* Berk. and Broome | | | 25.00 | 2.03 | 4.17 | 4.65 |
| 11. *G. fulvum* (Berke. and Broome) Trappe and Gerd. | | | | | 8.33 | 7.85 |
| 12. *G. intraradices* Schenck and Smith | 4.17 | 3.53 | 4.17 | 0.70 | 8.33 | 0.61 |
| 13. *G. microaggregatum* Koske, Gemma and Olexia | | | 4.17 | 0.36 | | |
| 14. *G. microcarpum* Iqbal and Bushra | 16.67 | 4.57 | 16.67 | 9.94 | 25.00 | 7.22 |
| 15. *G. mosseae* (Nicol. and Gerd.) Gerd. and Trappe | 12.50 | 3.24 | 8.33 | 5.43 | 12.50 | 16.88 |
| 16. *G. multicaule* Gerd. and Bakshi | 33.33 | 12.76 | 29.17 | 14.23 | 29.17 | 15.19 |
| 17. *G. rubiforme* Gerd. and Trappe | 58.33 | 10.38 | 41.67 | 12.02 | 29.17 | 6.73 |
| 18. *G. scintillans* Rose and Trappe | 4.17 | 2.78 | | | | |

Table 3: Continued

| | Study site | | | | | |
|---|---|---|---|---|---|---|
| | FORRU's research nursery | | Forest restoration plots | | Natural evergreen forests | |
| AM fungal species | F% | RA% | F% | RA% | F% | RA% |
| 19. *G. sinuosum* Gerd. and Bakshi | 4.17 | 0.35 | 4.17 | 0.33 | 16.67 | 2.88 |
| 20. *G. tortuosum* Schenck and Smith | 4.17 | 1.42 | | | | |
| 21. *G. viscosum* Nicol. | 8.33 | 2.78 | 8.33 | 0.51 | | |
| ***Scutellospora*** | 12.50 | 3.47 | 50.00 | 7.05 | | |
| 22. *S. gregaria* (Schenck and Nicol.) Walker and Sanders | | | 4.17 | 1.01 | | |
| 23. *S. heterogama* Walker and Sanders | | | 8.33 | 0.50 | | |
| 24. *S. pellucida* (Nicol. and Schenck) Walker and Sanders | 12.50 | 3.47 | 41.67 | 5.53 | | |
| Species richness | 17.00 | | 21.00 | | 15.00 | |

Table 4: Arbuscular mycorrhizal species and species richness associated with different herb species at two study sites

| | Study sites | |
|---|---|---|
| | Degraded watershed area | Forest soil extraction area |
| Herb species | Species[a]; (Species richness)[b] | Species[a]; (Species richness)[b] |
| *A. conyzoides* | 6, 17; (2) | 2, 6, 17; (3) |
| *A. margaritacea* | 18; (1) | 6, 17; (2) |
| *C. sumatrensis* | 6, 18; (2) | |
| *C. crepidioides* | 6; (1) | |
| *C. cyperoides* | 6, 14, 17, 22; (4) | |
| *E. adenophorum* | | 5, 6, 7, 17; (4) |
| *M. vagans* | | 2, 5, 7, 14, 17; (5) |
| *M. villosus* | 13, 6; (2) | |
| *P. aquilinum* | 6, 17; (2) | 17, 20, 23; (3) |
| *S. paniculata* | | 14, 17; (2) |
| *T. latifolia* | 14, 17; (2) | |
| Species richness | 6 | 8 |
| Average species richness | 2.00 | 3.17 |

[a]: No. of column refer to the codes of AM fungal species in Table 3, [b]: No. of brackets refer to the number of AM fungal species observed per rhizosphere of each herb species

100 g dry soil, with an average of 59.7 (Table 1 and 2). Average spore density at the FS, FN, FR and NF were high 79.7, 48.0, 97.4 and 57.4, respectively while the lowest spore density at the DW was 16.1. At the degraded watershed area, average spore density was significantly the lowest while it was highest at the forest restoration plot. Spore density in the rhizosphere of each plant species was highly variable, ranging from 4.0 to 595.7 spores per 100 g dry soil. The spore numbers of AM fungi at the study sites varied from 129 to 2,337 spores (Table 1 and 2). The spore numbers in the soils at the FN, FR and NF were high (1,152, 2,337 and 1,376, respectively). Much lower numbers were found at the DW and FS; 129 and 479, respectively.

Similarly, species richness of AM fungi at the study sites varied from 6 to 21 species per soil sample (Table 3). Species richness in the soils at the FN, FR and NF were also high (17, 21 and 15, respectively) and were lower at the DW and FS (6 and 8, respectively) showing trends with positively related to spore numbers. Species richness of AM fungi in the rhizosphere of each plant species was highly variable, ranging from 1 to 8 species per soil samples (Table 4 and 5). Average species richness of AM fungi in the soils ranged from 2.0 to 3.2 per soil samples, with an average of 2.8. In the rhizosphere of indigenous trees at the FN, RF and NF, species richness and spore number were higher than for ground herbs at the DW and FS.

Table 5: Arbuscular mycorrhizal species and species richness associated with different tree species at three study sites

| | Study site | | |
|---|---|---|---|
| | FORRU's research nursery | Forest restoration plots | Natural evergreen forests |
| Tree species | Species[a]; (Species richness)[b] | Species[a]; (Species richness)[b] | Species[a]; (Species richness)[b] |
| *A. fraxinifolius* | 2, 6, 7, 16, 17, 23; (6) | 3, 4, 16, 17; (4) | 12, 14; (2) |
| *B. baccata* | 5; (1) | 14, 23; (2) | 2, 12; (2) |
| *C. acuminatissima* | 7; (1) | 1, 16; (2) | 8, 17; (2) |
| *E. subumbrans* | 15, 17; (2) | 2, 7, 13, 22; (4) | 4; (1) |
| *F. altissima* | 5, 6; (2) | 2, 6, 10; (3) | 17; (1) |
| *F. benjamina* | 7, 16, 17; (3) | 6, 22, 23; (3) | 7, 14, 16, 18; (4) |
| *F. glaberrima* | 5, 6, 7, 16; (4) | 17; (1) | 7; (1) |
| *F. hispida* | 6, 14, 19; (3) | 2, 5, 17, 20; (4) | 7, 10, 15; (3) |
| *F. racemosa* | 6, 7, 14, 17, 20; (5) | 2, 8, 15, 17, 18; (5) | 7; (1) |
| *F. subulata* | 1, 6, 7, 16, 17; (5) | 2, 15, 17, 23; (4) | 7, 14, 16, 17; (4) |
| *G. kerrii* | 7, 14, 16; (3) | 24; (1) | 15, 16; (2) |
| *G. arborea* | 6, 17; (2) | 2, 6, 14, 16; (4) | 7, 17, 18; (3) |
| *H. trijuga* | 7, 17; (2) | 2, 3, 8, 10, 23; (5) | 11; (1) |
| *H. dulcis* | 2, 3, 6,15; (4) | 2, 7, 16; (3) | 2, 15, 18; (3) |
| *M. denticulata* | 7, 17, 21; (3) | 9, 10, 17, 23; (4) | 7; (1) |
| *M. bombycina* | 2, 6, 7, 16, 18; (5) | 2, 10, 17, 23; (4) | 6, 7, 14, 17; (4) |
| *M. toosendan* | 4, 17; (2) | 2, 9, 23; (3) | 2; (1) |
| *M. baillonii* | 6, 17; (2) | 17, 20; (2) | 3, 7, 18; (3) |
| *N. javanica* | 5, 6, 17, 20, 23; (5) | 6, 16, 23; (3) | 7, 16, 17; (3) |
| *P. cerasoides* | 7, 16, 17; (3) | 7, 10, 14, 16, 17; (5) | 7, 9, 14, 17; (4) |
| *R. rhetsoides* | 2, 5, 6, 7, 14, 16, 17, 23; (8) | 2, 6, 14, 17; (4) | 6, 7, 14, 16; (4) |
| *S. rarak* | 15; (1) | 2, 23; (2) | 2, 6, 11, 16; (4) |
| *S. arboreum* | 6, 7; (2) | 10, 12, 23; (3) | 7, 16; (2) |
| *S. axillaris* | 6, 12, 17; (3) | 16; (1) | 7; (1) |
| Species richness | 17 | 21 | 15 |
| Average species richness | 3.21 | 3.17 | 2.38 |

[a]: No. of column refer to the codes of AM fungal species in Table 3, [b]: No. of brackets refer to the number of AM fungal species observed per rhizosphere of each tree species

## DISCUSSION

The results of our study on the 24 framework tree species and 11 dominant herb species in the seasonally dry tropical forests of northern Thailand showed that most plant species are highly colonized hosts of AM fungi. This reflects the mycotrophic nature of the plant species studied, the age of the sites and the ability of AM fungi in soils to colonize a wide range of host species. It has been reported that many tree species are highly dependent on AM fungi (Janos, 1980; Onguene and Kuyper, 2001) and most herbaceous weeds and grasses are associated with AM fungi (Murakoshi *et al*., 1998). There were many intraradical hyphae, arbuscules and vesicles in the fine roots. Mycorrhizal colonization percentages differed among plant species and among sites. Colonization percentages were uneven in framework tree roots belonging to all growth stages. Some tree species had high colonization percentages at all sites: *M. denticulata*, *N. javanica*, *M. toosendan*, *H. dulcis*, *H. trijuga*, *E. subumbrans* and *R. rhetsoides*. This showed that indigenous forest trees may have a strong dependency on AM, as all surveyed plants formed AM and their roots were intensively colonized. The only exception was *C. cyperoides*, which is considered to be non-mycorrhizal or rarely mycorrhizal (Muthukumar and Udaiyan, 2000). In this study, it was found that this species commonly formed AM, with low colonization percentages (<2.4%).

Twenty four AM species were found in the rhizosphere of the plant species at surveyed study sites. AM fungi belonging to the genera *Glomus* and *Acaulospora* were dominant. This fact must be related to their sporogenous characteristics, i.e., *Glomus* and *Acaulospora* species usually take a short time to produce small spores, compared with the large spores of *Gigaspora* and *Scutellospora* species

in the same environment (Hepper, 1984; Bever *et al*., 1996). *Acaulospora* species are often associated with acidic soils. Most of the soils in our study sites were acidic and this could explain our frequent detection of *Acaulospora*. Among these, *A. elegans*, *A. scrobiculata*, *G. ambisporum*, *G. microcarpum*, *G. rubiforme* and *G. sinuosum* were the most commonly encountered species. This suggests that these species have a widespread and broad host range.

Spore densities of AM fungi vary greatly in different ecosystem. Here, the average density varied from 16.1 to 97.4 spores per 100 g soil and the species richness ranged from 6 to 21. The results of the current work showed that spore density was not related to colonization levels and species richness when all the study sites were considered together. Of the 24 tree species studied, 16 species had higher colonization percentage, 10 species were colonized by more AM species and 13 species supported higher spore densities in the forest restoration plot than in the natural evergreen forest. This clearly demonstrates than the forest restoration techniques used by FORRU maintains or increases AM fungal communities in the rhizosphere of most tree species planted. This may also help to account for the very high growth rates recorded for these tree species after planting them out in degraded areas (Elliott *et al*., 2003). This indicates that local environmental conditions and host plant species in each study site override AM fungal colonization, diversity and spore production.

The Degraded Watershed area (DW) had the lowest species richness (6 species) and average spore density (16.1 spores per 100g soil) in the rhizosphere of 8 herb species. These results indicate the influence of disturbance on mycorrhizal fungi in this site. It is well-known that land disturbance reduce below-ground AM fungal communities, depending on the disturbance intensity (Allen *et al*., 1998; Korb *et al*., 2003). In the Forest Soil extraction area (FS), the rhizosphere of 6 herb species supported higher species richness and average spore density (8 species and 79.7 spores 100 g soil, respectively) than the DW, even though the number of herb species examined from the FS was less than at the DW. Some species, such as *M. vagans* and *S. paniculata*, had high spore densities of AM fungi. This was probably because the FS was surrounded by an undisturbed area that still supported some favourable host plants. These plants may directly influence the below-ground AM fungal community composition, because different plant species exhibit varied abilities to establish mycorrhizal associations and to benefit from them (Lovelock *et al*., 2003).

The diversity of the AM fungi and the abundance of each species in the rhizosphere of 11 herbs at the DW and FS were low; very low compared to the rhizosphere of 24 indigenous trees at the other three sites. In FORRU's research Nursery (FN), the rhizosphere of selected tree saplings supported high AM diversity (17 species) and average spore density (48.0 spores per 100 g soil), but only a few AM species (5 species) were probably derived from the forest soil that is included as a component of the nursery potting medium. Furthermore, the growing of many tree species in close proximity in the nursery, the practice of raising plants on the ground or the location of the nursery within Evergreen Forest (EGF) all potentially contributed to high AM diversity and spore density in the nursery.

Highest AM diversity (21 species) and average spore density (97.4 spores per 100 g soil) were found in the rhizosphere of planted trees in the Forest Restoration plot (FR). This may be the result of the plant growth stage and plant diversity in the plot. This contrasts markedly with nearby degraded areas which support few AM fungal spores. After planting with framework trees however the AM fungal community was re-established. Forest restoration by planting 24 framework species therefore clearly promoted re-establishment of below-ground AM fungi that were still present or provided host trees for spores dispersed into the plot from nearby forest and enhanced establishment of new residual AM fungi that were inside the potting medium of transplanted saplings.

A denser plant community helps the colonizing obligate AM fungi to spread extensively, with less propagules being lost to passive stochastic dispersal. Dense plant cover also produces a high litterfall and great amount of root biomass for maintaining a diverse AM fungal community (Friberg, 2001). Similarly, the rhizosphere of mature trees showed high AM diversity (15 species) and average

spore density (57.4 spores per 100 g soil) in the Natural evergreen Forest (NF). The density and diversity of AM fungi increased with increasing tree canopy cover especially in deciduous forest, because plants in this habitat may more effectively convert the higher interception of light into photosynthate which can then be directed to the roots, providing a food source for AM fungi (Koske, 1987). In contrast, almost all samples in this study site were collected in the EGF with much higher and denser tree canopy than that of the deciduous forest. Fewer AM fungal spores were observed with increasing canopy cover, because the trees grew close to each other, strongly limiting light penetration to the soil, reducing soil temperature and possibly limiting sporulation and colonizaion of AM fungi.

In addition, the knowledge provided here has practical implications to forest management and regeneration technologies as follows (1). Including forest soil with indigenous AM fungi in the potting medium mix, allows most framework tree species grown in nurseries to become AM associated. The association is maintained after planting out trees in deforested sites (2). Tree nurseries should be located within forest areas and deforested landscapes should retain at least some natural forest to provide a continuous supply of AM fungal spores (3). Using forest soil with indigenous AM fungi as inoculum is preferable to the introduction of commercial innoculant products containing exotic AM fungi for growing framework tree saplings. Based on these studies, forest soil with indigenous AM fungi in the potting medium mix are important for the establishment, growth and survival of framework tree saplings at trial plot that might possibly accelerate natural regeneration of forest ecosystems and encourage biodiversity recovery in a degraded watershed area in Doi Suthep-Pui National Park.

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## REFERENCES

Allen, E.B., E. Rincon, M.F. Allen, A. Perez-Jimenez and P. Huante, 1998. Disturbance and seasonal dynamics of mycorrhizae in a tropical deciduous forest in Mexico. Biotropica, 30: 261-274.

An, Z.Q., J.W. Hendrix, D.E. Hershman and G.T. Henson, 1990. Evaluation of the Most Probable Number (MPN) and wet-sieving methods for determining soil-borne populations of endogonaceous mycorrhizal fungi. Mycologia, 82: 516-581.

Bever, J.D., J.B. Morton, J. Antonovics and P.A. Schultz, 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J. Ecol., 84: 71-82.

Brundrett, M., N. Bougher, B. Dell, T. Grove and N. Malajczuk, 1996. Working with Mycorrhizas in Forestry and Agriculture. ACIAR Monograph 32, ACIAR, Canberra, Australia, pp: 374.

Elliott, S., P. Navakitbumrung, C. Kuarak, S. Zangkum, V. Anusarnsunthorn and D. Blakesley, 2003. Selecting framework tree species for restoring seasonally dry tropical forests in Northern Thailand based on field performance. For. Ecol. Manage., 184: 177-191.

FORRU, 2006. How to Plant a Forest: The Principles and Practice of Restoring Tropical Forests. Forest Restoration Research Unit. 1st Edn., Department of Biology, Faculty of Science, Chiang Mai University, Thailand, pp: 200.

Friberg, S., 2001. Distribution and diversity of arbuscular mycorrhizal fungi in traditional agriculture on the Niger inland delta, Mali, West Africa. CBM:s Skriftserie, 3: 53-80.

Goosem, S.P. and N.I.J. Tucker, 1995. Repairing the rainforest, theory and practice of rainforest re-establishment in North Queensland's Wet Tropics. Wet Tropics Management Authority, Cairns, pp: 71.

Hepper, C.M., 1984. Isolation and Culture of VA Mycorhizal (VAM) Fungi. In: VA Mycorrhizae. Powell, C.L. and D.J. Bagyaraj (Eds.), CRC Press, Florida, USA., pp: 95-112.

Janos, D.P., 1980. Vesicular arbuscular mycorrhizae affect lowland tropical rain forest plant growth. Ecology, 61: 151-162.

Koide, R.T. and B. Mosse, 2004. A history of research on arbuscular mycorrhiza. Mycorrhiza, 14: 145-163.

Korb, J.E., N.C. Johnson and W.W. Covington, 2003. Arbuscular mycorrhizal propagule densities respond rapidly to ponderosa pine restoration treatments. J. Applied Ecol., 40: 101-110.

Koske, R.E., 1987. Distribution of VAM fungi a long a latitudinal temperature gradient. Mycologia, 79: 55-68.

Lovelock, C.E., K. Andersen and J.B. Morton, 2003. Arbuscular mycorrhizal communities in tropical forests are affected by host tree species and environment. Oecologia, 135: 268-279.

McGonigle, T.P., D.G. Evans and M.H. Miller, 1990. Effect of degree of soil disturbance on mycorrhizal colonization and phosphorus absorption by maize in growth chamber and field experiment. New. Phytol., 116: 629-636.

Morton, J.B., 1988. Taxonomy of VA mycorrhizal fungi: Classification, nomenclature and identification. Mycotaxon, 37: 267-324.

Murakoshi, T., M. Tojo, C. Walker and M. Saito, 1998. Arbuscular myorrhizal fungi on adjacent semi-natural grasslands with different vegetation in Japan. Mycoscience, 39: 455-462.

Muthukumar, T. and K. Udaiyan, 2000. Arbuscular mycorrhizas of plants growing in the Western Ghats region, Southern India. Mycorrhiza, 9: 297-313.

Onguene, N.A. and T.W. Kuyper, 2001. Mycorrhizal associations in the rain forest of South Cameroon. For. Ecol. Manage., 140: 277-287.

Setiadi, Y., 2000. Mycorrhizal Seedling Production for Enhancing Rehabilitation of Degraded Forest in Indonesia. In: Forest Restoration for Wildlife Conservation. Elliott, S., J. Kerby, D. Blakesley, K. Hardwick, K. Woods and V. Anusarnsunthorn (Eds.), Chiang Mai University, Thailand, pp: 235-243.