INTRODUCTION

Shifting cultivation is the dominant form of traditional land management undertaken by upland forest farmers in the mountainous region of mainland Southeast Asia; including Cambodia, Laos, Myanmar, Thailand, Vietnam and the neighbouring Yunnan province of China (Stark, 2006; Rerkasem and Rerkasem, 1994). The shifting cultivation system in these countries involves clearing vegetation by slashing and burning methods (Cairns and Garrity, 1999) followed by the planting of crops and then allowing natural revegetation to occur. The rotational shifting cultivation practiced by Karen farmers at Huai Tee Cha village in Sob Moei district, Mae Hong Son province, northern Thailand is one major form of crop production common in the region (Rerkasem and Rerkasem, 1994). At Huai Tee Cha the fallow forest will be slashed and burned after 7 years of regeneration before growing the main crop, upland rice, together with over fifty species of swidden crops in the field (Rerkasem et al., 2002; Yimyam, 2006). Restoration of soil fertility and maintenance of rice yield has been attributed to the presence of dense stands of Macaranga denticulata, a fallow enriching species known locally as pada (Yimyam et al., 2003).

In farmers’ fields, much greater nutrient accumulation and higher rice yield have been shown to be associated with dense stands of pada in the fallow forests (Yimyam et al., 2003). This tree has been reported to be highly dependent on arbuscular mycorrhizal (AM) fungi (Youpensuk et al., 2004). It is well known that AM fungi are widespread and form endomycorrhizal associations with most plants. A few plant species do not form mycorrhizal associations including members of the
Brassicaceae, Proteaceae, Caryophyllaceae, Cyperaceae and Juncaceae (Newman and Reddell, 1987). Plants associated with AM fungi generally have better nutritional status and better growth than those that do not. Arbuscular mycorrhizal fungi increase growth of many plant species by enhancing nutrient uptake and resistance to drought stress, and increasing tolerance to some pathogens (Smith and Read, 1997). The most understood benefit of AM fungi is the uptake and transfer of nutrients N, P, K, Ca, Mg, Cu, Mn and Zn to the host (Marschner and Dell, 1994). Of these, P is of particular interest due to its soil chemistry and depletion in the rhizosphere.

In many regions of the world, P deficiency is the major growth limiting factor in crop production and this is also the case in northern Thailand, where most soil types in upland agriculture are acidic and have available soil P at only 2-4 mg kg\(^{-1}\) (by Bray II) (Yimyam et al., 2003). However, the Karen farmers at Huai Tee Cha successfully organize their land by using pada trees for improving soil fertility. Arbuscular mycorrhizal fungi have been shown to influence pada growth, at the seedling stage (Youpensuk et al., 2004). The roots of pada trees growing in farmers’ fields are heavily colonized with AM fungi, and the fungi appear to be highly diverse, with 29 species in 6 genera identified in one study (Youpensuk et al., 2004). The effect of AM fungi on pada is well established. However, the roots of upland rice growing in this shifting cultivation system have also been reported to be heavily colonized by AM fungi (Youpensuk et al., 2005). The extent that AM fungi contribute directly to the yield of upland rice and other food crops in the system, in addition to an effect through nutrient accumulation and recycling via pada, is unknown. Therefore, managing beneficial AM fungi so as to improve plant nutrient uptake resulting in increased plant growth may help farmers to increase their yields.
and decrease inorganic fertilizer inputs. In addition, AM fungi can also improve nutrient retention on site and enhance nutrient cycling which should improve the sustainability of upland agricultural systems. The purposes of this study were to examine the association of upland rice and other food crops with AM fungi in farmers’ fields and to evaluate the role of AM fungi on growth, yield and nutrient uptake in these food crops experimentally. In addition, the role of AM fungi on upland and lowland rice were examined. Finding out how food crops respond to AM fungi would be useful to other farmers who operate similar and other systems of crop production on soils of low fertility in this and other countries.
1.1 Upland agriculture in northern Thailand

Upland area in northern Thailand covers 90% of the Upper North, which consist of Chiang Rai, Chiang Mai, Mae Hong Son, Lamphun, Lumpang, Phayao, Phrae, Nan, and Tak (Rerkasem and Rerkasem, 1994). Most of the people in upland areas belong to ethnic minority groups such as the Akha, Hmong, H’tin, Karen, Klamu, Lahu, Lisu and Lua, but there are also lowland Thai people who moved to the uplands (Bass and Morison, 1994; Rerkasem, 2003). The Karen people make up the largest share of the total population living in the forest and protected areas (Rerkasem, 2001). Shifting cultivation is the dominant form of traditional land management undertaken by upland forest farmers in Thailand and also in other Mainland Southeast Asia (MSEA) lands such as Cambodia, Laos, Myanmar, Vietnam and the neighbouring Yunnan province of China (Stark, 2006; Rerkasem and Rerkasem, 1994). In Southeast Asia, shifting cultivation is practiced widely and is often blamed for deforestation, weed invasion and erosion. Multi-purpose livestock are integrated with cropping in small-scale, mixed farming systems that characterize Asian agriculture (Devendra and Thomas, 2002). The shifting cultivation system in this region involves clearing vegetation by slashing and burning methods (Cairns and Garrity, 1999) followed by the planting of crops and then allowing natural revegetation to occur. This practice is also called swidden agriculture. There are
many forms of shifting cultivation, however, the traditional shifting cultivation may be conveniently divided into two different types, “rotational” and “pioneering” shifting cultivation (Rerkasem and Rerkasem, 1994).

1.2 Land management/land use/cropping systems

Kunstadter et al. (1978) divided shifting cultivation into three major types according to the management of fallow periods. These are: 1) short-cropping and short-fallow periods, often used by Northern Thai; 2) short-cropping and long-fallow periods, often used by Karen and Lua; and 3) long-cropping and long-fallow periods, often used by Hmong and other opium-growing hill tribe groups. Since the Thai Government has implemented policies for forest protection and watershed conservation, cash cropping was promoted to replace opium cultivation. The overall highland social development and opium-eradication strategy was formulated to settle ethnic minorities in permanent sites. The development of markets for highland products, the openings of roads, transport and other infrastructure have led to changes in shifting cultivation. From the development, the farmers can now sell their products in the cities or markets. Moreover, the provision of marketing and credit assistance from agro-companies, such as those dealing in fertilizers, herbicides, insecticides and seed germplasm, is a strong force leading to changes in shifting cultivation systems in Thailand, Vietnam and in Lao PDR (Bass and Morrison, 1994). Many parts of the uplands, throughout the Mekong region, are agronomically suitable for even more diverse crop production, including off-season and high-value commercial crops and including temperate vegetables and cut flowers as well as subtropical fruits for lowland markets and export. Agribusiness companies are having increased influence
on upland farming systems in many parts of the Mekong region such as in maize and soybean production for Thailand in Lao PDR and Myanmar, eucalyptus plantations for the Chinese pulp industry in Thailand, and rubber plantations for China in the Wa area of Myanmar (Rerkasem, 2003). Recently, traditional shifting cultivation is becoming rare and permanent cropping has become the major type of land use. Farming systems are converting to mono cropping and the purpose of crop growing for agribusiness. This type of upland agribusiness has been described for Mae Rit Pha Kae village in Mae Hong Son province (Public Welfare Department, cited by Yimyam, 2006). The farmers there have grown cash crops such as cabbage, pepper or tomato for external markets. In addition, they have grown upland rice and paddy rice as terraced fields for their staple food. Recently, Mong people living in Pah Poo Chom, Chiang Mai province of northern Thailand have turned to alternative cash crops, cabbage and lichee. They also grow upland rice and swidden crops such as maize, waxy corn, sweet sorghum and many local vegetables and root crops (Rerkasem, 2003). However, where market access is limited, pressure on the land nevertheless comes from government conservation policies as well as population growth. As illustrated by the case study of Huai Tee Cha village in Mae Hong Son, shifting cultivation to produce upland rice and other food crops is still the main mode of livelihood.

1.3 Upland agriculture in Huai Tee Cha village (a case study)

The Karen ethnic group making up Huai Tee Cha village, Sob Moei district, Mae Hong Son province still perform the rotational shifting cultivation or swidden agriculture system for food and some cash crops (Rerkasem and Rerkasem, 1994). In
this village, until very recently, the external pressure has been relatively small, inconsistent and temporary. However, traditional shifting cultivation has been under many pressures and the production system has been reduced. Since 1994 a fallow period at this village reduced to a shorter rotation, from 10-20 years to 7 years (Yimyam et al., 2003; Yimyam 2006).

1.3.1 Land management/cropping

The traditional shifting cultivation of Karen farmers in Huai Tee Cha village falls into the category of short-cropping and long-fallow periods (Kunstadter et al., 1978). The proportion of land use in 2004 in 3 major zones was: 1) natural forest zone 51.48%, 2) village site 0.51% and 3) agriculture zone 48.01%, total area being 1082.32 hectares. The agricultural zone was separated into three types: 45.79% in shifting cultivation (7 year crop rotation), 1.49% in permanent fields and 0.74% in paddy fields and fish ponds (Yimyam, 2006). Rotation of land use for agricultural practice in Huai Tee Cha village is shown in Figure 1.1. Briefly, after crops are harvested, the land is left for forest regeneration for six years, and at the 7th year of fallow period, the land is prepared once again for cropping. For land preparation, first the trees are felled and the branches and foliage are left on the ground to dry until they are ready for burning. Cropping patterns are based on rice as the main crop under rain-fed condition. Other swidden crops are planted together in the rice fields or along field edges just before the onset of the wet season (early May). Many swidden crop species are grown in the field before or after upland rice or mixed with the rice seed and sown at the same time (Yimyam, 2006). Land use differs between land holders of each farmer. Those who have large areas can delineate some parts of fields for growing high value cash crops such as chili, coffee, passion fruit, etc. Rice is not
only grown in under rain-fed conditions but is also grown in as paddy on terraces in a valley. There are total 17 name species were grown in upland, consisting with 12 varieties of non-glutinous and 5 varieties of glutinous type. A household would grow 3-5 upland rice varieties, depending on the conditions of the field and their preference. Less than 1% land use was paddy rice and 3-4 varieties (different from upland variety) were grown in the paddy field (Yimyam, 2006).

1.3.2 Possible roles of fallow trees

Farmers at Huai Tee Cha village have been using Macaranga denticulata (Bl.) Muell. Arg., as a fallow-enriching tree species, known locally as pada (Yimyam et al., 2003). Burning of vegetation releases nutrients previously stored in the forest biomass back onto the soil surface and makes it available for the crops during the cultivation period and also helps to neutralize the acid soil. The successful management of these local fallow species by farmers was established when the upland rice grown after dense pada patches had higher grain yield and grain N content compared with rice produced where pada had been sparse (Yimyam et al., 2003).
Figure 1.1 Rotation of shifting cultivation of Huai Tee Cha village

Source: Yimyam, 2006
1.3.3 The relations between plants and arbuscular mycorrhizal (AM) fungi in this system

Plants associated with AM fungi generally have better nutritional status, particularly phosphorus and better growth than those that do not (Smith and Read, 1997). The benefits of AM fungi are well known, including enhanced uptake of water and soil nutrients such as N, K, Ca, Mg, Cu, Mn, Zn and especially P, a nutrient which is often depleted in rhizosphere soil solution (Marschner and Dell, 1994). The association between AM fungi and pada was studied by Youpensuk et al. (2003) who established it to be highly dependent on AM fungi as they strongly increased growth and nutrient contents especially when the soil phosphorus supply was limiting. Moreover, the AM fungi population in the farmer's field appears to be highly diverse, with 29 species in 6 genera being collected in one study (Youpensuk et al., 2004).

Figure 1.2 The response of *Macaranga denticulata* to AM fungi under pot conditions; without AM (left) and with AM fungi (right)
1.4 Mycorrhizal fungi

1.4.1 Mycorrhizas

Mycorrhizas are the mutualistic associations between the fungus and the host plant. Mycorrhizas are the most widespread associations between microorganisms and higher plants. The roots of most soil-grown plants are usually mycorrhizal but certain taxonomic units; families such as Brassicaceae, Proteaceae, Caryophyllaceae, Cyperaceae and Juncaceae are largely non-mycorrhizal (Newman and Reddell, 1987). The host plant receives mineral nutrients while the fungus obtains photosynthetically derived carbon compounds (Harley and Smith, 1983). Mycorrhizal associations are classified according to the way in which fungi interact with a host plant root. They are categorized into seven main groups according to their morphology and on the basis of the fungal and plant taxa forming the symbiosis. The associations are: endomycorrhizas or arbuscular mycorrhizas (AM), ectocycorrhizas (ECM), Orchid mycorrhizas, ericoid mycorrhizas, ectendo mycorrhizas, arbutoid mycorrhizas, and monotropoid mycorrhizas (Brundrett et al., 1996). Among these symbioses, AM are by far the most abundant.

1.4.2 Arbuscular mycorrhizal (AM) fungi

The AM belong to the class Zygomycte order Glomales, is grouped in the phylum Zygomycota (INVAM website). It is characterized by the formation of branched haustorial structures (arbuscules) within the cortex cells and by a mycelium which extends well into surrounding soil (external hyphae, extraradical mycelium). Many, but not all of the endomycorrhizal fungi form vesicles as lipid-rich storage organs, hence it was formerly known as vesicular-arbuscular mycorrhizas (VAM). It is possible to identify individual Glomalean fungi by recognising characteristic root
morphology patterns in roots (Abbott, 1982). Identification of endophytes within roots is important for culture quality control, because contaminating fungi can be identified months before they sporulate (Brundrett et al., 1996). The best way to identify Glomalean fungus colonization in roots is to get to know them by examining single-isolate pot cultures. Mycorrhizal morphology is also influenced by host root structure, so it is best to work with one plant species. It is usually easier to identify fungi in roots with a thick cortex than in species with narrow roots. It is generally easy to recognize genera of AM fungi by their root colonization patterns, but it is also sometimes possible to separate species (especially within Glomus). Morphological features that are important include variations in vesicles (size, shape, wall thickness, wall layers, position and abundance), hyphal branching patterns, the diameter and structure of hyphae (especially near entry points), and the staining intensity of hyphae.

The symbiosis between an AM fungus and a plant host occurs as follows: spore germination, hyphal branching, appressorium development after contacting the root, colonization of the root cortex, formation of intracellular arbuscules, and concomitantly, production of an extraradical mycelium from which spores are eventually formed (Smith and Read 1997; Figure 1.3).
Figure 1.3 Scheme of the different stages of root colonization by an arbuscular mycorrhizal fungus (Balestrini and Lanfranco, 2006)
1.4.3 Classification of Glomales (INVAM website)

The classification of the Glomales is based largely on the structure of their soil-borne resting spores or morphology. At present, molecular techniques are alternative favor to identify and classify AM fungi. Nowadays, slightly over 180 AM fungal species have been identified and described and it was forecasted that the list of AM species may reach over 200 species by 1990 (Krishna, 2005). Glomus is thought to be the most abundant genus of the soil fungi (Lamont, 1982 referred by Marschner, 1995; Schwarzott et al., 2001). The classifying of Glomales fungi is provided in following:

Kingdom: Fungi
Phylum: Zygomycota
Class: Zygomycetes
Order: Glomales
Suborder: Gigasporineae
Family: Gigasporaceae
Genus: Gigaspora, Scutellocpora
Suborder: Glomineae
Family: Glomaceae
1.5 Factors affecting arbuscular mycorrhizal fungi

Mycorrhizas are three-way associations involving the host plant, the fungus and soil factors (Brundrett et al., 1996). A wide range of environmental, host plant, soil conditions, and other fungi may influence the symbiosis (Smith and Read, 2000). Many factors can affect AM root colonization and sporulation. For example, soils in low-input agricultural systems have larger populations of AM fungi than soils under conventional management or that are intensively disturbed. Both soil fertility and soil pH affect spore production of AM fungi and thus indirectly influence AM survival. In general, root colonization is less in low than in high pH soil (Clark, 1997). In pot culture with sorghum, intense AM root colonization and sporulation were found in the ranges of 5.6 to 7.1 for pH and 0 to 13 mg P kg\(^{-1}\) soil for available soil P (Friberg, 2001). In cowpea the percentage of root colonized by *Glomus etunicatum* was significantly lower than those colonized by *Gigaspora margarita* and decreasing the pH from 5.2 to 4.7 depressed the ability of *G. etunicatum* to colonized plant roots, but
did not affect *Gi. margarita* (Rohyadi *et al.*, 2004). The ability of *G. monosporum* to produce spores depends on the host plant species moreover, time or season and host age can influenced the mycorrhizal population as well (Husband *et al.*, 2002; Youpensuk *et al.*, 2004).

Application of nitrogen and phosphorus together strongly depressed root colonization and spore density of AM fungi (Youpensuk *et al.*, 2004). Both phosphorus and nitrogen reduced root colonization if present at high level; and application of nitrogen fertilizer not only reduced root colonization but suppressed spore formation (Smith and Read, 1997). In a maize cropping system, Na Bhadalung *et al.* (2005) found that long-term NP- fertilization decreased AM fungi spore abundance and variation in species diversity depended on sampling time. Very high and very low phosphorus levels may reduce mycorrhizal infection or colonization (Koide, 1991). At high soil phosphorus levels, infection by mycorrhizal fungi is generally reduced (Amijee *et al.*, 1989; Koide and Li, 1990) and there may be a delay in infection as well as a decrease in the percentage of root infection (deMiranda *et al.*, 1989). Abbott and Robson (1979) concluded that levels of soil phosphorus greater than that required for plant growth eliminated the development of the arbuscules within the host plant cells. Ratnayake *et al.* (1978) and Graham *et al.* (1981) demonstrated that the net leakage of root exudates is significantly greater under low phosphorus levels. High levels of exudation were correlated with decreased phospholipids levels and increased permeability of root membranes. They suggested that root colonization by AM fungi is inhibited at high phosphorus levels because of the decreased root exudation. Phosphorus deficiency in the host increased appressorium formation and, therefore, enhanced mycorrhiza development in onion (Tawaraya *et al.*, 1998). The
presence or absence of a host plant obviously plays a large role in whether or not colonization and subsequent sporulation will occur. Karasawa et al. (2002) found growth and root AM colonization of maize increased when grown following sunflower (mycorrhizal plant) compared with maize following mustard (non-mycorrhizal plant). Degree root colonization of crop reduced when it was grown following non-mycorrhizal plant (Hayman et al., 1975; Gavito and Miller, 1998). In addition, AM fungi abundance and effectiveness are declining with land use intensity and continuous mono-cropping (Oehl et al., 2003). Corn grown in no tillage had greater root colonization than in the tilled treatment (Galvez et al., 2001). Likewise, Kabir et al. (1997) compared three tillage practices on corn and found that densities of total and metabolically active soil hyphae, and mycorrhizal root colonization were lower in conventional tillage soil than in reduced tillage and no tillage soil. Examining of relative preference of perennial ryegrass and white clover for co-existing with AM fungi inoculation, the roots of white clover were more highly colonized than ryegrass (Zhu et al., 2000)

1.6 The benefits of AM fungi to host plants

Arbuscular mycorrhizal fungi are normally considered to improve plant mineral nutrition, in particular phosphorus, water uptake, and resistance to root pathogens (Smith and Read, 1997). The implication of this on global nutrient cycling and agriculture is very large since more than 80% of land plants form associations with AM fungi (Newman and Reddel, 1987). Generally, vascular plants absorb inorganic nutrients from soil solution but mycorrhizal symbiosis may provide access to additional nutrient sources through hyphal enzyme activity or by other physical or
chemical modification of the rhizosphere (Marschner, 1995). In turn, the host plant provides both carbohydrates and lipids to the fungus (Koide, 1991; Smith and Read, 1997) mainly due to the capacity of the mycorrhizal fungi to absorb phosphate from soil and transfer it to the host roots (Asimi, et al., 1980). Arbuscular mycorrhizal fungi can play an important role for plant nutrient uptake, especially on soils with low phosphorus availability. AM fungi usually increase the growth of plants by enhancing nutrient uptake. Total uptake of P, K, Ca, Mg and Zn in sorghum and cowpea were increased 2.5 to 6 fold by AM fungi (Bagayoko et al., 2000). Inoculated rice seedlings produced higher biomass at maturity under field condition than those not inoculated and grain yield was increased 14-21% (Solaiman and Hirata, 1997). In addition, mycorrhizal infection has been reported to increases the uptake of copper (Gildon and Tinker, 1983), zinc (Lambert et al., 1979), nickel (Killham and Firestone, 1983), chloride and sulphate (Buwalda et al., 1983). Mycorrhizae also are known to reduce problems with pathogens which attack the roots of plants (Gianinazzi-Pearson and Gianinazzi, 1983). Colonization of tomato root by Glomus mosseae compensated for the reduction of plant growth by Meloidogyne incognita infection (Talavera et al., 2001). Peanut plants inoculated with AM fungi show tolerance to the nematode and offset the growth reduction caused by Meloidogyne arenaria (Carling et al., 1996). Moreover, AM fungi can alleviate problems from high soil As level in maize plants (Bai et al., 2008). Arbuscular mycorrhizal fungi can enhance seedling vigor of many trees for instance, cashew inoculated by G. faciculatum had greater shoot length, internode number, internode length, number of leaves, stem diameter, root length and root number than uninoculated plants (Ananthakrishnan et al., 2004). Mycorrhizal infection enhanced in biomass
production, relative growth rate and leaf area of 75 days old pioneer seedling: *Ipomoea wolcottiana* (Huante *et al*., 1993). Height, dry weight, and nutrient content of fallow enriching tree: *Macaranga denticulata* was enhanced by AM fungi as well (Youpensuk *et al*., 2004).

1.7 Variation in response to AM fungi within and among plant species

The benefits or dependency of plants to symbiosis with AM fungi are usually measured as the percent increase in yield, biomass, nutrient uptake, etc. of inoculated plants compared to uninoculated plants (Plenchette *et al*., 1983). Highly mycorrhizal dependent plant species derive more benefits from AM fungi in comparison with plant species with a low mycorrhizal dependency (Van der Heijden *et al*., 2003). Different plant species growing under standard soil conditions show large differences in responsiveness to AM colonization (Jakobsen *et al*., 2002). Moreover, mycorrhizal dependency can be influenced by soil type, soil phosphorus and the AM fungi. Different dependencies to AM fungi have been reported among plant species (Plenchette *et al*., 1983; Schweiger *et al*., 1995; Monzon and Azcón, 1996; Duponnois *et al*., 2001), and cultivars (Azcon and Ocampo, 1981; Baon *et al*., 1993; Diop *et al*., 2003). Mycorrhizal dependency varies between crop species and varieties, for different levels of available soil nutrients, particularly phosphorus and zinc and among the different AM genera and species. From the selection of efficient AM fungi for wetland rice, Secilia and Bagyaraj (1992) found that *Glomus intraradices* and *Acaulospora* sp. were suitable for inoculation into rice nurseries.

Nwoko and Sanginga (1999) calculated mycorrhizal dependency (MD) of ten recent selections of promiscuous soybean breeding lines by using formula of
Plenchette et al. (1983), and separated in three groups as follows: 1) the highly dependent plants, 2) the intermediate group, and 3) the majority of soybean lines that were not mycorrizal dependent. Mycorrhizal dependency of twelve species of tropical legumes varied from 26.6% to 92.7% when inoculated with *Glomus aggregatum* (Duponois et al., 2001). The percentage root colonized by AM fungi was different between different wheat cultivars that varying from 16% to 37% (Zhu et al., 2001). Mycorrhizal dependency of shoot growth ranged from 73 to 95% among cultivars of welsh onion (Tawaraya et al., 2001).

Vandenkoornhuyse et al. (2002) studied the diversity of AM fungal community composition in the roots of *Agrostis capillaries* and *Trifolium repens* by using molecular techniques. They found that, 19 of phylotypes belonged to the Glomaceae, 3 were Acaulosporaceae and 2 were Gigasporaceae and demonstrated that the AM fungal community colonizing *T. repens* differed from colonizing *A. capillaries*, providing evidence for AM fungal host preference.

Most studies of symbiosis between AM fungi and crop plants have been on lowland crops especially those grown using modern agronomic practices. The principles developed there need extending to be applied to more traditional genotypes grown under upland agricultural conditions. Important cause of land degradation is soil infertility that causes slow regeneration of forest species and low crop yield. The low crop yield, in turn, leads to more extensive use of land more forest encroachment. Information about identifying key processes that maintain and improve soil fertility will help to reduce pressure on the land and lessen degradation and may provide useful information to other farmers who operate similar and other systems of crop production in this and other countries.