

Agroecological Practices for a Sustainable Tea Production in Northern Vietnam

by

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Agroecological Practices for a Sustainable Tea Production in Northern Vietnam



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Abstract

Tea is a very important cash crop in Vietnam, providing crucial income and employment for farmers in poor rural areas. Unfortunately, the dominance of long-term, conventional tea cultivation, which strongly relies on agrochemical inputs has caused severe soil health degradation, low tea productivity and quality as well as human health concerns and environmental pollution. At the same time, as tea production may provide a better net income compared with other annual crops such as rice and vegetables, farmers have been converting parts of their allocated land to cultivate tea plants. Little is known about the benefit of agroecological management as an alternative to conventional tea management practices, and thus, there is a need to understand how it can improve tea yields, quality, and the livelihoods of the farmers. In addition, since soil acidification has been one of the major concerns of the tea industry in Vietnam as well as in the world, cost-effective strategies such as liming, the application of agricultural wastes and byproducts have been promoted to control soil acidification, restoration and maintain soil fertility and biodiversity. Among these solutions, the role of liming in ameliorating soil acidity and enhancing soil health and crop productivity is widely documented although poorly understood in the case of tea plantations in Northern Vietnam.

In this study, we investigated the sustainability of agroecological tea production along with the main challenges of conventional tea farming in Northern Vietnam, the mechanisms and consequences of tea soil acidification and soil health degradation; the potential uses of agricultural wastes/composts and liming for managing soil acidification and subsequently improving tea soil health- related properties while enhancing crop productivity and quality. A total of 66 different tea households were selected for assessing the economic efficiency, and afterward, 20 tea farms from these households were selected for field experiments and sampling, including both agroecological and conventional plantations, as well as converted and non-converted fields. Soil physical and chemical attributes, soil

fauna, and tea root AMF, as well as tea yield and yield components, were analyzed to compare the impacts of agroecological and conventional tea management methods. To assess the effect of the 9-month liming application on tea soil biodiversity, soil bacterial, fungal, and AMF community richness, and composition were also determined using rDNA and ITS gene sequencing analyses, in addition to soil physicochemical and fauna assessments.

Our study showed that apart from potentially bringing about production economic efficiency due to cheap inputs and high productivity in the short term, the continuity of conventional tea production in Vietnam has led to a series of severe issues, including soil degradation, particularly soil acidification, poor tea quality, environmental pollution, and human health concerns. Agroecological tea management practices such as the application of organic fertilizers, biofertilizers and biopesticides, organic mulching, intercropping as well as integrated pest/disease management have been widely reported to retain soil physicochemical and biological attributes. These beneficial impacts are mainly driven by the additions of organic matter and soil essential macro and micronutrients, which enrich soil organism diversity and functional activities, as well as reduce the use of agrochemical inputs and chemical residues in soil and on tea leaves. Converting conventional tea adoption to agroecological management practices significantly increased tea root AMF intensity by up to 24%, soil macro, and mesofauna by 110% and 60%, respectively, and soil pH by 0.5 units on average. Despite the lower tea yields, our study indicated for the first time that agroecological tea adopters earned around USD 8,400 ha/year more than the farmers still practicing conventional management, which was mainly driven by the premium price of agroecological tea products and other credits from supporting agencies.

Though tea soil acidification has numerous consequences on soil chemical and biological properties, as well as tea quality and productivity, in which the reduction and imbalance of nutrient base cations, including Ca^{2+} , Mg^{2+} , Na^+ , and K^+ have been considered one of the most serious

disadvantages, this issue can be mitigated by the applications of liming and/or agricultural wastes and byproducts, which could supply alkaline matter and essential elements to neutralize soil acidity, thus improving soil health-related properties and crop performance. Consequently, 9 months after the application, liming significantly enhanced soil pH (by 0.4 units) and soil OM, while strongly reducing soil exchangeable Al^{3+} and Mn^{2+} , and P availability. Converting paddies and vegetable fields to tea farms also resulted in higher soil pH values, OM, and soil P availability, but also increased soil Al toxicity risk. Macrofauna observed in tea soils was less abundant than in organic mulch, and liming also had a significant and positive effect on macrofauna abundance recorded in these layers. The lime amendment also significantly enhanced tea AMF intensity and frequency, as well as tea yield and yield components, regardless of the land use history difference. In contrast, soil bacterial, fungal, and AMF relative abundance and composition were strongly responsive to land use history, and the interaction of liming and land use history, because of changes in soil physicochemical properties and crop types. The sole lime application did not lead to any significant impacts on soil microbial richness or community composition, indicating that a 0.4-unit shift in soil pH may not be enough to trigger a significant change in soil microbial communities. Additionally, lime incorporation created a better environment for the growth of some fungal taxa, while suppressing other fungal groups which are preferable to acidic soils, thus fungal diversity appeared to be unaffected by liming. Since the single liming application from this study was not enough for inducing a significant and positive effect on diversity and composition of soil microbial communities, further studies might be needed to investigate other liming strategies, such as frequency and application rates, sampling depth and study period. How lime addition affects other organisms (nematodes, soil microfauna, soil microbial functional diversity) also deserves further investigation to provide a better understanding of liming efficiency in enhancing the food web in tea plantation soils.

Our findings contribute towards understanding changes in soil biodiversity in response to liming and land-use conversion and confirm that appropriate liming could be an effective strategy to ameliorate soil acidity, thus enhancing soil biodiversity and crop productivity. Additionally, this study highlights the important roles of soil microbial communities concerning tea quality indicators and other aspects of tea plantation management in ensuring that suitable and sustainable management practices are promoted for restoring soil fertility in the region. The strategies developed in this study might also be useful in understanding and improving the sustainable management of other regional perennial crops, such as coffee and fruit orchards. Where applicable, the promotion of agroecological farming and other soil acidification management strategies could benefit both the local population and the environment through a reduction of expensive agrochemical inputs and an increased source of income.

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1. CHAPTER 1: General introduction

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See the “Authorship Statement – Chapter 1” (appendix 1) for details.

Tea (*Camellia sinensis* Kotze) is one of the oldest beverages in the world. In 2017, global tea production amounted to nearly 6 million tons, of which 2.1 million tons were exported worldwide (Tuan 2018; Voora et al. 2019). In the same year, the global tea market was valued at approximately USD 50 billion. This economic sector is projected to grow from 4.5 to 5.3 per cent annually to reach USD 73 billion in 2023. Globally, the top five tea producing countries in 2017 were China, India, Kenya, Sri Lanka and Vietnam, while the top five tea exporters were Kenya, India, Sri Lanka, Argentina and Vietnam (FAO 2018; International Institute for Sustainable Development 2018). In 2016, globally the total area of registered organic tea was around 542,000 hectares (ha), which was 13,2% of the total global tea production area (Willer and Lernoud 2019).

Vietnam has been cultivating tea for thousands of years. In Vietnam, tea is one of the most important cropped plants, both for domestic consumption as well as export. The current area of tea plantations in Vietnam is around 130,000 ha, with the total production volume of fresh tea leaves being over 1 million tons (Bui and Nguyen 2020; Doanh et al. 2018). Tea is mainly grown in the Northern mountainous and Central Highland provinces. It plays an essential role in providing a livelihood and economic sustainability for these regions (Cong Bien et al. 2018; Doanh et al. 2018). The tea industry employs around 1.5 million people. Since 2010, the annual economic contribution of tea exports has been over USD 200 million (GSO 2020a; Hong and Yabe 2015; Van Ho et al. 2019).

Tea cultivation in Vietnam has been dominated by conventional management practices. The application of chemical fertilizers and pesticide have long been a tradition of tea growers to improve tea productivity, reduce pest and disease damage and maintain soil fertility (Doanh et al. 2018; Phong et al. 2015a). Common tea production practices also include mono-cropping and cultivation of tea on steep sloping land (MARD 2016; Toan and Phuong 2014). Combined, these conventional management practices have resulted in soil degradation and erosion, reduced tea productivity and

quality, and led to increasing concerns as to environmental problems and human health impacts (Hong and Yabe 2015; Van Ho et al. 2019). Although the production and export volume of Vietnamese tea over the last two decades has consistently increased, due to the low-quality, Vietnamese tea products have been mainly exported to lower-value markets such as Taiwan, Pakistan, Iran, Indonesia and Russia (Doanh et al. 2018; Van Ho et al. 2019).

Vietnamese tea export prices have been consistently lower than the world average. From 2012 to 2019, despite rapid growth in the Vietnamese tea export volume and the markets, Vietnamese tea price per kg was 35-40% below the world average pricing (GSO 2020a; Khoi et al. 2015). The main reason for the reduced price is the high proportion of chemical residues, which results in limited market access due to non-compliance with international regulations such as those of the European Union (Doanh et al. 2018; MARD 2017). In addition, poor post-harvesting technology and lack of branding development further exacerbate the poor market price (Khoi et al. 2015). Over the past decade, there has been an increasing demand for tea quality standards such as pesticide residues limits, hygiene and contaminants of the international market, and Vietnam recently has joined numerous international trade agreements such as the Trans-Pacific Partnership (TPP) and the Regional Comprehensive Economic Partnership (RCEP). Subsequently, Vietnamese tea producers could lose not only the international markets but also their home market to other exporters such as China and India without improvements in tea quality and safety (Xiong 2017).

The quest for sustainable tea production and higher quality is driving an increasing conversion from conventional tea farming practices to organic-based methods; where non-chemical pest and disease management practices are used (Ha 2014a; Hong and Yabe 2015; Van Ho et al. 2019). This transformation in tea management practices has been driven by the growing interest in high quality and economic efficiency of tea production, and increased awareness of the harmful effects of agrochemicals on human health and the environment (Doanh et al. 2018; Ha 2014a). In addition,

supporting policies and programs from the Vietnamese Government and international agencies are also playing an essential part in promoting the conversion and convincing the Vietnamese tea producers to implement more agroecological practices (Ha 2014a; Van Ho et al. 2019).

Agroecology is the application of natural ecological system processes and concepts for optimizing the interactions between humans, plants, animals and the environment, whilst also considering the social aspects to ensure a fair and sustainable food system (FAO 2020). Agroecology can play a vital role in supporting food production, nutrition improvement, and food security while restoring ecosystem services and biodiversity, which are important to sustainable agriculture (Chappell and LaValle 2011; FAO 2020). In addition, agroecology provides a practical way for restoring soil quality depleted by conventional management practices (Altieri et al. 2020).

For tea production, numerous studies conducted outside of Vietnam have indicated the beneficial impacts of agroecological management practices such as using organic fertilizers (Li et al. 2015; Lin et al. 2019), biofertilizers (Nepolean et al. 2012; Roychowdhury et al. 2014; Xu et al. 2014), biopesticides (Nakai 2014; Roychowdhury et al. 2014), mulching, intercropping (Jianlong et al. 2008; Sun et al. 2011; Zhang et al. 2017) and integrated pest and disease management strategies (Mamun and Ahmed 2011; Shrestha and Thapa 2015). These practices can result in soil health improvement (biological, chemical and physical properties), and reduce agrochemical input and chemical residues in soil and on tea leaves. Ultimately, agroecological tea production practices can mitigate the negative effects of chemical uses on the environment while maintaining tea productivity and quality (Gui et al. 2021; Han et al. 2018). In Vietnam, the benefits of agroecological tea management, assessing profitability and social and policy aspects have been investigated to a limited degree (Doanh et al. 2018; Duc and Goto 2019; Van Ho et al. 2019). Previous studies have also examined the impacts of mulching and biofertilizers on soil quality (Cu and Thu 2014a, b) but

to our knowledge, there has not been any study investigating the effects of other agroecological practices such as organic fertilizers, intercropping or non-pesticide pest and disease management.

Based on this, our chapter will (1) determine the challenges and positive impacts of conventional tea management systems in Vietnam, and (2) evaluate the potential benefits of agroecological tea management practices on soil health, tea quality, and then (3) recommend the most suitable management and supporting policies for enhancing and sustaining tea production without negative impacts on the soil and its environment.

1.1 Tea plantations in Vietnam

Tea plants (*Camellia sinensis* Kotze) belong to the Theaceae family and are native to East Asia. However, these perennial plants have now been cultivated all around the world, in tropical and subtropical areas (Meegahakumbura et al. 2018). In Vietnam, there are two main kinds of tea plants: wild tea and domesticated cultivars. The wild tea is referred to as Shan tea, which is mainly grown in the Northern high mountainous provinces (above 1000 m) such as Ha Giang, Lai Chau, Yen Bai, Lao Cai and Dien Bien (Hatvala 2018). Wild tea can reach 15 meters in height, and differ with the domesticated varieties in terms of growing, cultivation and processing (Intellectual Property Office of Vietnam, 2018). The domesticated tea varieties are small woody plants or evergreen bushes, with pointed and fragrant leaves. Both these kinds of tea plants are cultivated in 28 out of 64 provinces in Vietnam, and the five largest tea producers in the nation are Thai Nguyen, Ha Giang, Phu Tho, Lam Dong and Tuyen Quang provinces (GSO, 2020b). Of these five provinces, four are in the North while Lam Dong is in the Central Highland (Fig. 1). Currently, there are around 230 different tea varieties being cultivated in the country, of which some high yield and quality varieties such as LDP1, LDP2, PH8, PH11... have been widely used (Hung et al. 2019; NOMAFSI, 2021). Different

periods involved in the production of these varieties, from seedlings to mature plantations, are summarized in Fig. 2.

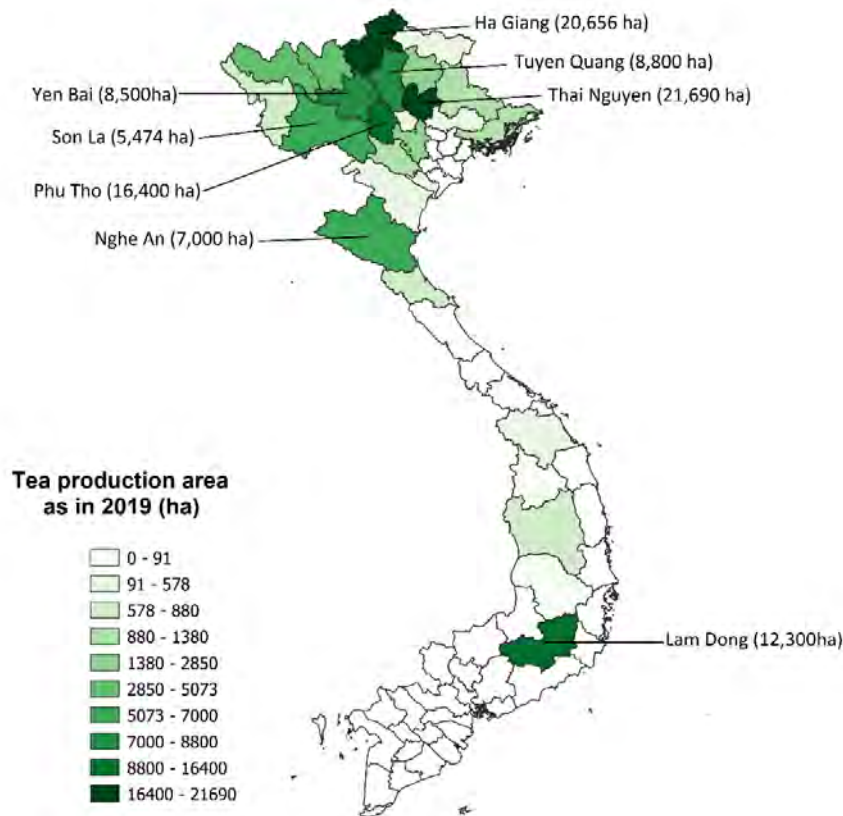


Figure 1. Distribution of tea plantations in Vietnam in 2019. Data was sourced from GSO (2020b), Ha Giang government (2019) and Thai Nguyen government (2019)

Beginning with the “Doi Moi policy” in 1986, a transformation that aimed to gradually improve the economic efficiency towards ‘a socialist market economy under state guidance’ (Beresford 2008), the land allocation policy and the issuance of long-term land use right certificates to households were implemented nationwide, aimed at strengthening farmer's decision-making capacity to boost production and encourage the protection of natural resources (Saint-Macary et al. 2010). Since then, tea production has contributed significantly to economic development and social sustainability of the country; especially in rural regions where livelihood for farmers has been limited (Khoi et al. 2015). Vietnam was one of the seven largest tea exporters worldwide from 2010 – 2019 (top five

from 2010- 2017) and in return, exports of tea products contributed around USD 200 million annually to the country’s economic revenue (GSO 2020a; Van Ho et al. 2019). It is also forecasted that Vietnam will remain as one of the top five tea producers and exporters globally, and become the second largest green tea exporter after China by 2027; with projected export volumes of around 148,500 tons (FAO 2018).

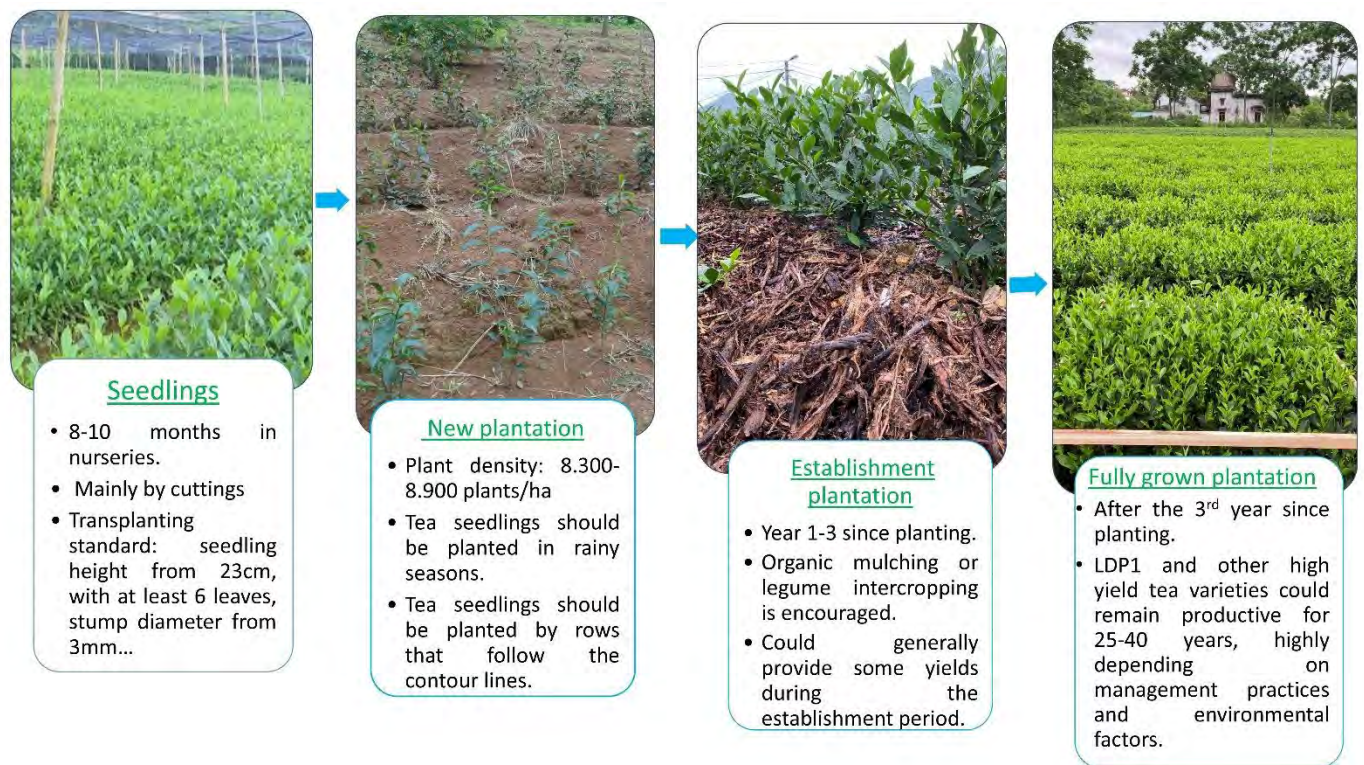


Figure 2. Summary of the main periods of tea growing timeline, from seedling to mature plantations of high yield and quality varieties such as LDP1, LDP2, PH8, PH9 and so on

The tea industry in Vietnam provides employment to more than 400,000 small households in rural regions and 600 industrial tea processing companies. Annually, around 1,5 million people are employed in tea production, processing and other related fields of the tea value chain such as trading and services. Aside from its economic and social importance, tea also plays a critical role in Vietnamese culture, as it has been used as a daily beverage in every part of the country for centuries (Wenner 2011).

1.2 Soil health and conventional tea management

1.2.1 Soil health and tea soil in Vietnam

Since the concept for soil quality was introduced in the early 1990s, numerous revisions have been proposed (Bünemann et al. 2018; Karlen et al. 1997) and the term ‘soil health’ is now more frequently used than ‘soil quality’ (Bonfante et al. 2019). Simply, soil health can be defined as the capability of a soil to provide ecosystem services (Williams et al. 2020). From an agricultural perspective, soil health refers to the capacity of soil to support crop productivity (Mursec 2011). Soil health is now acknowledged to consider all the soil feature indicators including soil physical, chemical and biological properties (Allen et al. 2011; Arias et al. 2005; Cardoso et al. 2013). In recent decades, agricultural soil has been seriously degraded by human interventions (Mursec 2011; Shah and Wu 2019). Conventional agricultural practices such as agrochemical applications and mechanical tillage have led to significant soil health degradation, including reduced soil biodiversity (Alori et al. 2020). For the tea farming industry, long-term conventional tea cultivation has been considered as the main driver of severe soil acidification, soil nutrient imbalance and nutrient leaching in tea plantations (Li et al. 2016; Yan et al. 2020).

In Vietnam, the requirements for soil characteristics used for tea plantations were issued by the Ministry of Agriculture and Rural Development (MARD) under the technical procedure for Tea production: 10TCN446:2001 (Chung 2013). This technical document lists soil features for optimizing tea growing and productivity: a well-drained soil with a pH (KCl) of 4 - 6, soil depth and organic matter of at least 50 cm and 2% respectively, and soil slope of less than 25 degrees were recommended. However, in Vietnam tea is grown in 28 distinct provinces where soil characteristics, climatic and topography vary significantly, and for many areas the soil physical and chemical properties do not meet the MARD requirements. For instance, about 60% of tea producing districts of Phu Tho province typically grow tea in soil containing less than 2% of organic matter, and on

steep sloping land (slope >25 degrees) (MARD 2016; Tea Research and Development Center 2015). In addition, tea plants need an adequate amount of both macronutrients (nitrogen, phosphorus and potassium) and micronutrients (zinc, boron and aluminum) for optimal growth and productivity (Hajiboland 2017).

1.2.2 Conventional tea management practices in Vietnam

Intensive use of agrochemicals are the common practices of tea growers worldwide (Sitienei et al. 2013; Wu et al. 2016a; Xie et al. 2021). Among the chemical fertilizers, nitrogen (N) is widely used to ensure high tea productivity. In Japan, tea fields are amended with the N application rate of more than 1000 kg/ha/year (Abe et al. 2006; Zou et al. 2014). In China, the N application rate can reach 1200 kg/ha/year. This amount far exceeds the actual uptake of N by tea plants (Wu et al. 2016; Yan et al. 2020). Vietnamese tea farmers traditionally apply mineral fertilizers either multiple times after each harvest or 1- 2 times a year during rainy seasons, after tea pruning. The rate of fertilization application varies widely between provinces or regions and is based predominantly on individual farmer decisions and their experiences. The recommended application rate of fertilization for tea cultivation in Vietnam is 300 kg N + 150 kg P₂O₅ + 150 kg K₂O per ha. However, tea growers generally exceed the recommendation of the manufacturers to ensure a satisfying tea growth, yield and soil nutrient loss replacement (Hong et al. 2016; Huu Chien et al. 2019). Since 2000, Vietnam has imported 3.5 - 4.5 million tons of inorganic fertilizers per year, with Vietnamese farmers spending on average around USD 5 billion per annual on fertilizers (Toan et al. 2019).

Around 50 different pests and insect species are known to cause damage to parts of the tea trees, especially to young tea leaves and tea buds, resulting in significant tea yield losses (Tu 2019). In 2015, more than 71,000 ha of tea plants were damaged by tea pests, such as *Empoasca flavescens* (green leafhopper), *Helopeltis theivora* (tea mosquito bug), *Physothrips setiventris* (tea thrips) and *Oligonychus coffeae* (red spider mite) (MARD 2016). In addition, leaf and stem diseases have been

commonly found in most of tea plantations (Hung and Tao 2006) while root diseases caused by fungal species such as *Poria hypolateritia* (black root rot) and *Poria hypolateria* (red root rot) are among the most destructive tea diseases in Vietnam (Phong et al. 2015b). Tea yield losses caused by pests and diseases ranged from 10 to 15% on average but can be up to 100% in severe conditions (Phong et al. 2015b; Shrestha and Thapa 2015).

To reduce the incidence of these pests and diseases, widespread application of pesticides and fungicides have been the dominant practice. Vietnamese tea farmers use an estimated amount of 128 liters of pesticides per ha annually (Hong et al. 2016). Apart from chemical pesticides, in recent years alternatives practices such as biological pest management (alcohols, local herbs, light trap and manual removal) and biopesticides have also been applied to protect tea fields (Thu 2016; Tu 2019) (Table 1). However, the proportion of farms using non-chemical pest and disease management methods is still very low compared to chemical use (NOMAFSI 2015; Thu 2016). In 2019, the market value of biopesticides was estimated at USD 31 million. This equates to only 3% of the total value of the plant protection market in Vietnam (Mordor Intelligence 2019).

Table 1. Summary of the most common tea pest and diseases and its control methods in Vietnam. Data was retrieved from MARD (2001), Hung and Tao (2006), Thu (2016), Tu (2019) and other unpublished data

Common name	Scientific names	Classification	Chemical pesticides (*) (Active ingredients)	Biopesticides (**) (Active ingredients)	Other non-pesticide methods (**)
Blister blight	<i>Exobasidium vexans</i>	Leaf disease	Imibenconazole	Abamectin; bacillus bacteria, yeast	Alcohols, botanicals, host plant resistant, pruning
White blight	<i>Phyllosticta theaefilia</i>	Leaf disease	Imibenconazole	Abamectin; lactic acid bacteria, yeast	Alcohols, botanicals, host plant resistant, pruning
Horsehair blight	<i>Marasmis equimidis</i>	Leaf disease	Mancozeb 80%	Abamectin; bacillus, yeast	Alcohols, host plant resistant, pruning, botanicals
Grey blight	<i>Pestalotiopsis thea</i>	Leaf disease	Chlorothalonil; Difenoconazole,	Abamectin; bacillus bacteria	Alcohols, host plant resistant, pruning, botanicals
Damping off	<i>Ustilina vulagrini</i>	Root disease	Mancozeb + Metalaxyl	<i>Trichoderma</i> spp. <i>Bacillus</i> spp.	Manual removal, host plant resistant
Black root rot	<i>Rossellinia arcuata</i>	Root disease	Fosetyl aluminium, Phosphorus acid	<i>Trichoderma</i> spp. <i>Actinomyces</i> spp.	Manual removal, host plant resistant
Red root rot	<i>Poria hypolateraria</i>	Root disease	Mancozeb 80%	<i>Trichoderma</i> spp. <i>Chaetomium</i> spp.	Manual removal, host plant resistant
Brown root rot	<i>Fomes noxius</i>	Root disease	Fosetyl aluminium, Phosphorus acid	<i>Trichoderma</i> spp. <i>Chaetomium</i> spp.	Manual removal, host plant resistant
Green leafhopper	<i>Empoasca flavescens</i>	Tea pest	Bup rofezin; Cartap	<i>Beauveria bassiana</i> ; Abamectin;	Plucking, predators, field sanitation
Tea mosquito bug	<i>Helopeltis theivora</i>	Tea pest	Bup rofezin; Etofenprox	<i>Beauveria bassiana</i> Matrine; Isoparafin	Plucking, predators; botanicals, field sanitation, light trap
Tea thrips	<i>Physothrips setiventris</i>	Tea pest	Etofenprox	Abamectin; Isoparafin; Azadirachtin	Plucking, predators; botanicals, field sanitation, light trap
Leafroller	<i>Gracillaria theivora</i>	Tea pest	Emanectin Benzoat	Abamectin; Matrine; Azadirachtin	Plucking, predators, field sanitation
Red spider mite	<i>Oligonychus coffeae</i>	Tea pest	Acrinathrin; Propagite	Matrine; Azadirachtin	Plucking, light trap, predators

Note: While the chemical pesticides (*) are commonly used in conventional tea management practices, biopesticides and other non-pesticide methods (**) are the main pest and disease control methods in agroecological tea farming in Vietnam.

1.2.3 Agrochemical overuse and its effects on soil health

For tea and most other plant crops produced in Vietnam, conventional agriculture employing intensive usage of chemical fertilizers has been considered as one of the main causes of soil health degradation (Kundu et al. 2016; Suhag 2016). This farming approach brings about economic efficiency due to cheap inputs and high productivity in the short term but results in the degradation of soils and leads to reduced tea productivity in the long run (Doanh et al. 2018). Long-term use of mineral fertilizers in tea cultivation can result in problematic soil acidification. A field study by Chong et al. (2008) found that long-term conventional tea cultivation resulted in soil pH of 3.38 compared to 4.16 of organically managed tea soil (sampling was conducted at depth of 0-20cm). Likewise, 70% of studied conventional tea fields that applied intensive calcium cyanamide (CaCN_2) had soil pH values below 4.0, of which 9% were below 3.0 (Oh et al. 2006). Lin et al. (2019) argued that excessive chemical usage in tea cultivation resulted in nutrient imbalance and increased heavy metal levels in soil which exacerbates soil acidification. Using only compound fertilizers in conventional tea production resulted in the highest loss of soil fertility and degradation compared to the combination of organic manure and mulching; indicated by the decreases of soil health indicators such as wet stable aggregates, soil organic matter, soil organic carbon and the increases of soil bulk density and soil penetration resistance (Yüksek 2009). High levels of heavy metals (Cu, Ni, Zn, Hg, As, Cd, Cr and Pb) and pesticide residues (imidacloprid, β -*/Hexachlorocyclohexane, permethrin) were also recorded in tea plantations subjected to continual over application of agrochemicals (He et al. 2020). Likewise, various pesticide residues such as Ethion (47,75 ppb), Chlorpyrifos (177,75 ppb); Heptachlor (115,05 ppb) and Dicofol (187,70 ppb), were detected in soils of conventional tea plantations in India. Comparatively, in soils from organic tea plantations, levels of the aforementioned heavy metal contaminants and residual agrochemicals were all below the detection levels (Bishnu et al. 2009).

Although there is no study on the effects of heavy pesticide applications on tea plantations in Vietnam to date, investigations on other crops in Vietnam and other countries showed that long-term pesticide use can have negative impacts on soil quality properties. A 10-year study conducted from 2002-2013 on vegetable production in Vietnam concluded that about 80% of pesticides have been used incorrectly (e.g. violated with the “4 Rights” principles and/or did not comply with the preharvest intervals) (Galli et al. 2022; Hoi et al. 2016). This has resulted in increased production costs and environmental pollution. Also, residues of many pesticides including dichlorodiphenyltrichloroethane (DDT), dicofol and isoprothiolane have been found in soil samples from the Red River Delta of Northern Vietnam (Nishina et al. 2010). The World Bank warned that land and soil pollution in Vietnam, caused mainly by fertilizer application and pesticide residues from farming activities, is a significant emerging problem (Nguyen 2017). Burrows and Edwards (2002) showed that increased pesticide use negatively affected microbial activity and diversity of nematodes and earthworms. A reduction in the populations of fungal, actinomycete and protozoal populations due to the applications of Edosulfan and Pyrethroid was also found (Kalia and Gosal 2011). Similarly, fungicides (Bavistin and Dithane M-45) and carbendazim led to an extensive reduction of many soil fungi species in first 20 days, such as *Penicillium* spp, *Mucor racemosus* and *Aspergillus ruber* (Aggarwal et al. 2005). Heavy uses of chlorpyrifos, carbendazim, 2,4-D and carbofuron in paddy fields resulted in a significant decrease of soil bacterial populations and soil microbial biomass (Arora et al. 2019). Similar findings on the negative effects of pesticide applications on soil organisms were also reported by Arora and Sahni (2016), Kalia and Gosal (2011), and Lo (2010).

1.2.4 Soil erosion

Aside from soil and vegetation characteristics, inappropriate farming practices such as mono cropping, over ploughing, burning, or clearing out plant residues and pesticide applications have

been considered as the main causes of soil erosion in Northern Vietnam (Alam 2014; Vezina et al. 2006). Soil erosion is a major cause of soil degradation in Vietnam; affecting about 40% of Vietnam's total land surface area (Ha et al. 2012; Phuong et al. 2014). As 80% of tea plantations in Vietnam are grown in high rainfall, mountainous regions on steep sloping land (Doanh et al. 2018), soil erosion is a constant and difficult challenge. Studies on the effects of slopes on soil erosion rates in Northern Vietnam have indicated that soil loss occurs at a rate of 96 tons/ha/year at a 3-degree slope, 211 tons/ha/year at an 8-degree slope and can reach 305 tons/ha/year at a slope of 15 degrees (Nguyen and Pham 2018).

Tea seedlings are commonly grown at a recommended spacing of 100 cm by 60 cm and this leaves a large area of soil open to surface erosion, especially during the crop establishment period when tea canopy is not yet closed. Sahoo et al. (2016) showed that rainfall runoff observed in tea fields without soil conservation measures in the establishment years ranged from 30 to 35%. Similarly, Zhang et al. (2003) estimated that soil loss by erosion from tea plantations in China could be up to 4,000 tons/km²/year. Soil erosion leads to soil organic matter and nutrient losses, reduced soil water holding capacity, exposure of subsoil with high acidity and poor fertility, thus resulting in soil health degradation (Lal et al. 2018; Zheng-An et al. 2010).

1.2.5 Environmental and human health concerns

To date, the effects of extensive pesticide applications in tea cultivation with negative consequences on the environment and health of Vietnamese tea farmers and consumers have not been clear. Scientific investigations carried out in other farming activities have indicated a strong alarming signal to this concern. The study of Dasgupta et al. (2007), investigated the levels of agrochemicals and pesticides on 190 rice farmers in the Mekong Delta region of Vietnam. The results from this study showed a high prevalence of pesticide poisoning by organophosphate and carbamate exposure with over 35% of test subjects experiencing acute pesticide poisoning (Dasgupta et al. 2007). At

national level, more than 3,000 cases of pesticide poisoning were recorded by the Ministry of Health, causing more than 100 deaths in Vietnam just in the first half of 2011 (Dang et al. 2017). Research on tea plantations from other Asian and African countries also concluded that intensive usage of pesticides and fungicides puts the health of tea growers and consumers at risk (Hajiboland 2017). Using the modified QuEChERS method, a recent study conducted in China found 102 different pesticide residues in tea leaves (Huang et al. 2019). Likewise, Feng et al. (2015) found 198 out of 232 harvested tea samples of green tea, oolong tea and black tea were contaminated with pesticide residues, and the residue levels in 39 samples were exceeding the maximum residue limits of the European Union. Globally, pesticide poisoning is a public health issue, which has been responsible for around 300,000 deaths worldwide every year (Sabarwal et al. 2018).

1.3 Advantages of conventional tea management method

Comparative analyses as to the benefits of conventional tea farming in Vietnam versus other cultivation alternatives is largely undocumented. Studies conducted in other tea growing nations have indicated this farming method could have some positive benefits in comparison with organic and other tea management methods. Intensive applications of chemical fertilizers generally increase tea yield, compared to the yields from plants grown solely supplemented with organic fertilizers (Das et al. 2016; Yang et al. 2012). Many agrochemicals such as fertilizers and pesticides commonly used by conventional tea farmers are typically cheaper and more readily available, especially in the remote and mountainous regions of Vietnam (Doanh et al. 2018). In addition, conventional tea producers also do not need to seek certifications. Tea producers that adopt agroecological management strategies must meet the criteria of compliance watchdogs, including organic, VietGAP and the Rainforest Alliance. Demonstrating compliance can be both costly and time consuming (Ha 2014; Van Ho et al. 2019). Finally, conventional farming practices typically have lower labor input requirements, which is an advantage that convinces many tea growers to stick with the conventional

management approach (Doanh et al. 2018; Qiao et al. 2016).

1.4 Agroecological management practices for sustainable tea development - A promising approach

1.4.1 Agroecological tea production in Vietnam

Traditionally in Vietnam, agroecological practices such as manure and plant residue applications, intercropping, mulching and agroforestry have been utilized in a range of cropping systems including maize, vegetables, forests and fruits (Dzung et al. 2013; Nguyen and Pham 2018). In recent years, other farming management systems and practices including organic agriculture, VietGAP standards, integrated pest and/or disease management (IPM or IDM), biofertilizers and biopesticides use have been promoted – mainly in rice, vegetable, fruit, tea and coffee plantations (Doanh et al. 2018; Duc and Goto 2019). The VietGAP refers to a voluntary standard package providing the criteria and requirements for safe and sustainable agriculture production, enhanced by certification and auditing processes (Nicetic et al. 2016; Van Ho et al. 2019). To be certified, VietGAP adopters must record all practices on their farms, from field selection, to production, harvest and processing. VietGAP products also need to meet the pesticide limits (Nicetic et al. 2016). In addition to VietGAP standards, organic farming also has received increasing attention. In 2010, there were 21,300 ha of certified organic agricultural land in Vietnam, accounting for about 2% of total agriculture land areas (Nguyen Dang Nghia 2016). By 2016, this had grown to 77,000 ha (Suharyono 2018).

In the tea production sector, there has also been increased interest in and implementation of agroecological management practices in recent years; in form of organic and other cultivation methods (Doanh et al. 2018; Ha 2014a). In 2019, the provinces with largest certified VietGAP and organic tea areas are Ha Giang, Phu Tho and Thai Nguyen with nearly 7,000 4,100 and 1,600 ha of

tea plantations respectively (Ha Giang Government 2019; Thai Nguyen Government 2019). Other agroecological practices such as intercropping with legumes in the establishment years (1-3 years old), incorporation of shade trees, organic mulching, terraces and mini terraces have been also practiced, either separately or in combination (NOMAFSI 2015). Currently, the proportion of certified VietGAP and organic tea areas is still very small compared to the total area of tea production (Thai Nguyen Government 2019). However, this figure is expected to rapidly grow in the next few years as many tea producers are currently adopting VietGAP and organic management practices (Ha Giang Government 2019; Thai Nguyen Government 2019). At a national level, more than 52,000 ha of tea plantations were proposed to meet the VietGAP standard by 2020, accounting for around 42% of the total tea plantations in the country (Thu 2016).

1.4.2 Potential advantages of agroecological tea cultivation

Soil physical and chemical properties

The influence of agroecological tea management on soil physical properties has been well documented. A study conducted in tea fields in Vietnam showed that tea residue mulches application used in combination with biofertilizers containing various beneficial microorganisms (*Bacillus* spp., *Lactobacillus* spp., *Streptomyces* spp., *Saccharomyces* spp.) resulted in a significant decrease of soil bulk density and an enhancement to soil moisture content (Cu and Thu 2014b). Likewise, using the fern *Gleichenia linearis* as mulch improved soil moisture when applied at a rate of 25 tons/ha. Sun et al. (2011) argued that straw and plastic mulches increase soil water content and water use efficiency, while Peng et al. (2006) indicated that straw mulches and intercropping with legumes stabilizes tea plantation temperatures, reducing the deleterious impact of high temperatures and daily temperature fluctuations of soils. Organic fertilizers such as sheep manure significantly improved soil porosity, soil bulk density and particle density (Chepkorir et al. 2018).

In tea cultivation, organic fertilizer applications such as sheep manure significantly reduced soil

acidification and improved soil N content (Chepkorir et al. 2018; Li et al. 2018). Studies have shown that phosphate solubilizing bacteria (PSB) and arbuscular mycorrhizal fungi (AMF) can convert nutritionally important elements from unusable to usable forms (Roychowdhury et al. 2014; Sabaiporn et al. 2020). Phosphate solubilizing bacteria such as *Bacillus* spp. and *Pseudomonas* spp. were shown to increase P availability to tea plants by converting P from insoluble to soluble forms to increase its availability resulting in increased plant growth (Gebrewold 2018). Mulching practices and intercropping with soybean resulted in a significant reduction of exchangeable aluminum (Al) content, weed occurrence, disease and pest incidences, but increases of soil pH, organic matter content and N content (Jianlong et al. 2008). Organic mulches (straw, chopped grass, legumes) have been shown to significantly increase soil organic matter and soil N status (Sun (Sun et al. 2011); soil organic carbon, soil pH, available P, and total N content (De Silva 2007). After adding tea pruning mulches and biofertilizers (*Bacillus* spp., *Lactobacillus* spp., *Streptomyces* spp., *Saccharomyces* spp.) to soils of tea plantations a significant increase in soil organic matter content, concentration of exchangeable cations (Al^{3+} , Ca^{2+} and Mg^{2+}) and a decrease of soil acidification was observed (Cu and Thu 2014b).

Soil biological properties

Agroecological tea management practices such as organic fertilizers, biofertilizer application and mulching are known to improve soil biological diversity and structure (Bishnu et al. 2009; Roychowdhury et al. 2014). The application of tea pruning mulch over soil surface as a single practice or combined with biofertilizers significantly increased the number of bacteria by up to ~1734,6%, actinomyces by ~319% and fungi by ~24,5% compared with non-mulch and biofertilizer treatments (Cu and Thu 2014b). A similar study done in China indicated that long-term application of rape cake and sheep manure significantly increased the abundance of many bacteria such as Burkholderiales, Streptomycetales, Acidobacteriales, Nitrospirales, Solibacterales and

Gemmatimonadales (Lin et al. 2019). Such increases of soil microbial abundance are important for tea plants, as an enhanced soil microbiome can incorporate potentially beneficial bacteria that enhance plant growth and productivity. For instance, *Nitrospira* spp. play a role in the nitrification processes of the N cycle (Koch et al. 2015; Lückner et al. 2010); *Burkholderia* spp. can improve plant growth as well as inhibit pathogen growth (Wu et al. 2016); and *Streptomyces* spp. could help to mitigate plant diseases as they are able to produce various bioactive metabolites, such as antibiotics, anti-inflammatory and antimicrobial enzymes (Kinkel et al. 2012; Lyu et al. 2017). Gu et al. (2019) compared chemical fertilizers and organic manure (cow and pig manure) combined with commercial organic fertilizers in tea plantations. Outcomes from Gu et al. (2019) study showed significant increases in the relative abundance of soil microorganism groups that were able to perform chemoheterotrophy (29%), N fixation (41%), fermentation (110%), and aerobic nitrite oxidation (557%) in the organic fertilizer treatments. Other studies focusing on mulching practices in tea gardens also indicated that organic mulch materials such as chopped grass and straw all increase microbial biomass carbon, population of beneficial fungi, bacteria and mycorrhiza (De Silva 2007).

At present, the impacts of agroecological tea management practices on soil microbial diversity and community structure are largely undocumented in Vietnam, but they have been well studied in other countries such as in China. Qiu et al. (2014) illustrated that organic manure application resulted in the highest microbial diversity in soils compared to NPK treatment only. Similarly, sheep manure was shown to mitigate soil acidification, enhance the diversity and abundance of soil microbes, as well as improve the overall microbial community structure in soils of tea plantations (Li et al. 2018). In terms of soil fauna, a study in China showed that organic tea cultivation led to an increase in common species diversity, species richness and trophic diversity of nematodes in both soil surface and subsurface layers in comparison with conventional tea farming (Li et al. 2014).

Tea quality, productivity, production cost and market development

Agroecological management practices have the potential to be more beneficial for tea quality than conventional management approaches by reducing or eliminating the agrochemical uses, thus reducing pesticide residues, as well as improving other tea quality indexes such as amino acid and water extract content (Birch et al. 2011; Reddy 2017). Some studies have indicated that organic fertilizers produced from animal manure and vinegar residues can significantly improve tea quality indicators (amino acids, water extract content and caffeine content) and reduce the heavy metal contents such as arsenic and cadmium in tea leaves as compared with mineral fertilizer applications (Li et al. 2015; Li et al. 2016). Likewise, the application of biofertilizers containing *Paenibacillus polymyxa* produced from effluent generated during sweet potato starch production, resulted in increased levels of tea polyphenol and water extract content by 10.4% and 6.3% respectively (Xu et al. 2014). The beneficial outcomes from other forms of agroecological cultivation on tea quality have also been reported such as mulching (Sun et al. 2011; Yüksek 2009), intercropping (Jianlong et al. 2008; Sedaghatoor and Janatpoor 2012), non-chemical pest and disease managements (Mamun and Ahmed 2011; Shrestha and Thapa 2015).

Compared to conventional fertilizer usage, the application of sole organic fertilizers and biofertilizers generally resulted in lower tea yields, especially in the transition period although the differences are not always significant (Doanh et al. 2018; Haorongbam et al. 2014; Lin et al. 2012b). However, field experiments suggest that combining organic fertilization and biofertilizers could lead to higher tea productivity compared with chemical fertilizers, particularly in the long run (Haorongbam et al. 2014; Lin et al. 2012b). Despite organic manure and biofertilizer have lower levels of nutrients than mineral fertilizers, the presence of growth promoting compounds such as enzymes, hormones, organic matter, as well as a higher microbial activity and functional diversity, could make them essential for the improving soil fertility and productivity (Haorongbam et al. 2014). To obtain optimal tea productivity and reduce the harmful effects of intensive mineral

fertilizer usage, a combination of organic fertilizer with proper proportion of chemical fertilizers has been widely recommended (Lin et al. 2012b; Qiu et al. 2014; Xie et al. 2019).

A higher income is one of the main driving factors for the conversion from conventional management to agroecological tea practices. Recent studies indicated that organic tea adopters could earn a better net income than their conventional counterparts (Deka and Goswami (Deka and Goswami 2021; Doanh et al. 2018). Although implementation of agroecological tea strategies generally requires extra investments for labor, relevant certifications and pests and diseases control, agroecological tea adopters typically invest less on agrochemicals (Bui and Nguyen 2020; Doanh et al. 2018; Qiao et al. 2016). In addition, agroecological tea products can be sold with higher prices as tea consumers seek for tea with a better quality (Bui and Nguyen 2020; Doanh et al. 2018). All these factors contribute to a significantly higher net income, especially in long-term agroecological managed fields.

In 2015, approximately 75,000 tons of registered organic tea were produced worldwide, valued at around USD 765 million. It is forecast that organic tea will experience a rapid growth of 6-13% per year due to the increasing demand for a chemical-free tea beverage by consumers (Hajra 2017). In 2017, the global tea market was valued at approximately USD 50 billion. It is further predicted this economic sector is projected to grow by around 5.3% annually to reach USD 73 billion in 2024 (Statista 2020). As one of the leading tea producers and exporters in the world, this market growth creates a great opportunity for the Vietnamese tea industry to expand their international market and improve the international market share of high-quality tea export. The beneficial impacts of agroecological tea management practices on aspects of soil health, tea quality and productivity are summarized in Table 2.

Table 2. Summary of the benefits of some agroecological management practices on tea soil properties, tea quality and productivity

Agroecological practices	Materials	Beneficial effects	References
Organic fertilizers	<ul style="list-style-type: none"> • Rape cake and sheep manure • Commercial farmyard manure made from chicken manure • Vinegar residue 	<ul style="list-style-type: none"> • Increased soil pH, the relative abundance of various bacteria; significantly increased the amino acid content but reduced the contents of Cadmium (Cd), Lead (Pb) and Arsenic (As) in tea leaves. • Resulted in the greatest levels of soil organic matter, total N, total P, N available, K available; richest soil bacterial community diversity compared to only chemical fertilizers and half chemical plus half organic manure applications. • Significantly increased alkali-hydro N, available P and available K compared to chemical fertilizers and tea quality indexes (tea amino acid, caffeine, water extract and polyphenols) 	<p>Lin et al. (2019)</p> <p>Qiu et al. (2014)</p> <p>Li et al. (2015)</p>
Biofertilizers	<ul style="list-style-type: none"> • Phosphate-solubilizing bacteria (PSB), arbuscular mycorrhizal fungi (AMF) and tea residues. • <i>Paenibacillus polymyxa</i> produced from wastewater from the sweet potato starch industry • Nitrogen fixing <i>Azospirillum</i>, phosphate solubilizing bacteria and AMF fungi. 	<ul style="list-style-type: none"> • Retained soil fertility and nutrient quality, mobilized nutritionally important elements from unusable to usable forms; enriched soil microorganisms, increase tea yield, and reduce chemical fertilizer usage. • Tea yield, level of tea polyphenol and water extract content were significantly increased by an average of 16.7%, 10.4% and 6.3% respectively. • Increased tea soil fertility, reduced tea disease and chemical fertilizers. 	<p>Roychowdhury et al. (2014)</p> <p>Xu et al. (2014)</p> <p>Nepolean et al. (2012)</p>
Biopesticides	<ul style="list-style-type: none"> • Insect-pathogenic fungus <i>Metarhizium anisopliae</i>. • Doubled stranded viruses of Baculovirade family • <i>Aspergillus niger</i>, <i>Azospirillum brasilense</i>, <i>Bacillus subtilis</i>, <i>Beauveria bassiana</i>, <i>Trichoderma harzianum</i>, <i>T. viride</i> and <i>Verticillium lecanii</i>. 	<ul style="list-style-type: none"> • Significantly reduced the incidence of adult <i>Aedes aegypti</i> and <i>Aedes albopictus</i> mosquitoes. • Successfully controlled <i>Adoxophyes honmai</i> and <i>Homona magnanima</i> species. • <i>A. niger</i> and <i>T. harzianum</i> are the most compatible microbe in relation with commonly used pesticides in tea (<i>Azadirachtin</i>; <i>Dicofol</i>; <i>Phosalone</i>; <i>Clothianidin</i>; <i>Deltamethrin</i> etc.), suggesting that there may be some compatible agrochemicals that can be utilized in combination with the biopesticides. 	<p>Roychowdhury et al. (2014)</p> <p>Nakai (2014)</p> <p>Dutta et al. (2016)</p>

Mulching	<ul style="list-style-type: none"> • Straw mulches • Chopped grass (<i>Brachia decumbens</i>); legume (<i>Calliandra calothyrsus</i>) • Straw mulch and white clover as living mulch 	<ul style="list-style-type: none"> • Resulted in an increase of soil water content and water use efficiency, significantly increased soil organic matter, available N, nitrate N, and ammonium N and tea yield (12-13%). • Improved soil pH, soil organic and microbial biomass carbon, soil CEC, plant available P, and total N contents. Grass and legume mulches increase the population of positive microorganisms (bacteria, fungi, and mycorrhiza). • Straw and white clover enhanced the stability of soil temperature in the same layer, decreasing daily temperature difference and the emergence of harmful high temperatures. 	<p>Sun et al. (2011)</p> <p>De Silva (2007)</p> <p>Peng et al. (2006)</p>
Intercropping	<ul style="list-style-type: none"> • Soybean (<i>Glycine max</i>) • Aromatic plants (<i>Cassia tora</i>, <i>Leonurus artemisia</i>, <i>Medicago sativa</i> and <i>Mentha haplocalyx</i>) 	<ul style="list-style-type: none"> • Significantly improved soil nutrient by reducing exchangeable Al content, increasing soil pH and organic matter, total N content and available N; reduce weed occurrence, disease and pest incidences. • Reduced the population of tea green leafhoppers, increased the natural enemies of tea pests such as spiders, coccinellids, lacewings, and parasitoids. 	<p>Jianlong et al. (2008)</p> <p>Zhang et al. (2017)</p>
Integrated Pest/Disease Management (IPM/IDM)	<ul style="list-style-type: none"> • IPM-FFS (Farmers Field School). IPM relied on local communities • IPM (light traps, heat treatment, manual removal, planting of rehabilitation and trap crops, pruning) 	<ul style="list-style-type: none"> • Enhanced safe tea production and improved tea farmers' income. • Significantly reduced the incidence of common tea pests such as termites, mosquitos, aphids, caterpillars, spiders and so on; reduce chemical uses, pesticide residues and improve tea quality. 	<p>Shrestha and Thapa (2015)</p> <p>Mamun and Ahmed (2011)</p>

Soil erosion reduction

Several agroecological management practices were found to effectively reduce soil erosion as well as improve water retention capacity of tea soil. Sahoo et al. (2016) indicated that cover crops, vegetative barriers, and contour staggered trenches significantly reduced rainwater runoff by 15-20% in the first two years of tea plantations. Similarly, Sharma and Gunasekare (2018) suggested that shade tree planting is an effective solution to mitigate soil erosion in tea soil as they prevent rainwater from directly contacting the ground and reduce rainwater speed. Vetiver grass (*Vetiveria zizanioides* L) also offers an effective break, reducing water runoff and preventing soil erosion in tea plantations on slopes (Haridas 2001). Practices such as intercropping, mulching, growing grass hedgerows, mini-terraces not only for tea but other plants like coffee, rubber and other annual crops in Northern Vietnam significantly reduced soil erosion and water runoff (NOMAFSI 2016). Organic manure (buffalo manure), vermicompost, earthworms (*Eisenia andre*), and compost uses in maize plantations in Northern Vietnam resulted in a significant reduction of runoff water, soil loss and leachate properties, in comparison with only chemical fertilizer treatments (Doan et al. 2015). The lowest amount of soil loss was recorded in the vermicompost treatments (0.3 kg/m²), followed by compost (0.4 kg/m²), organic manure (1.1 kg/m²) and the chemical fertilizer (1.4 kg/m²) (Doan et al. 2015). In comparison with traditional practices, the application of mulching and terraces in the Northern mountainous areas of Vietnam significantly reduced soil loss by ~90.3% and ~93.9%, respectively (Nguyen and Pham 2018). As 70% of Vietnam's tea production occurs on sloping land, of which 50% slope more than 20 degrees (Tien 2015), appropriate agricultural practices to reduce soil erosion and water loss is playing a key solution to restore and improve soil health (Lal 2015; Nguyen and Pham 2018).

1.5 Soil fauna in tea plantations

Soil fauna are a vital part of all soil types as they play a key role in altering and transporting soil components, particularly in organic matter decomposition and soil structure development as well as an important indicator of soil fertility (Dumanski, 2006; Cardoso et al. 2013; Stoops, 2018). Moreover, they are also key players in several supporting and regulating ecosystem services (Coleman and Wall 2015). Rough estimates of soil biodiversity indicate several thousand invertebrate species per site, as well as the relatively unknown levels of microbial and protozoan diversity (Menta 2012). Even though soil fauna species can be found in any parts of soils, the understanding of its quantity, functions and classification are not well covered (Dumanski 2006).

Soil fauna composition and structures are affected by numerous biotic and abiotic factors, ranging from the ecosystems that they live in, crop cultivation methods, to soil types and soil properties (Debeljak et al. 2007; Menta, 2012; Pulleman et al. 2012). For instance, Loranger-Merciris et al. (2007) argued that soil macrofauna abundance in the Vertisol of planted forest was significantly different compared to the natural forest Leptosol. In terms of cultivation aspects, many of the current agricultural management practices impact soil fauna communities, which could be translated to effects on key soil ecological processes such as nutrient or water cycles that crop production relies on (Domene 2016). A study conducted in Australia showed that different rotations such as sown pasture and alternate crop breaks significantly reduced the population of all known detrimental soil fauna associated with yield decline in sugarcane (Pankhurst et al. 2003). It is also noted that management practice and type of cultivation had more influence on soil fauna than different soil types (Fromm et al. 1993)

To date, several studies on tea soil fauna have been conducted worldwide. When extracted soil samples collected from tea soil of 0-15cm in depth, Li et al. (2018) found 23 soil fauna phyla

belonging to 10 different classes and 5 groups, in which Nematoda was the dominant group. Jamatia and Chaudhuri (2017b) also found 17 different earthworm species under tea plantation system in India while Senapati et al. (2002) indicated that intensive tea cultivation could deplete soil fauna diversity as well as soil fertility, but these depletions could be recovered by the application of organic materials.

In Vietnam however, research on tea soil fauna has received very limited attention. Presently, there have been two studies which analyze the impacts of some organic material application on tea soil organisms, but they focus only on some soil microbes and did not take soil fauna into account (Cu and Thu, 2014a; Tu, 2019). To have a better knowledge on the effects of agroecological approach on tea soil fauna to promote this farming approach, a better understanding of the effects of this on soil fauna is critical.

1.6 Plant - microbe interaction

Soil is a dynamic natural environment of plant–microbe interactions (Bhattacharyya and Jha, 2012; Farrar et al. 2014) and is known to trigger the production of plant growth hormones which are antagonists of plant pests and pathogens, in addition to harnessing essential micro- and macronutrients that affect plant growth (Benizri et al. 2001). Beneficial microorganisms include a diverse array of the soil microbiota, such as rhizobia, mycorrhizal fungi, actinomycetes, diazotrophic bacteria, which create opportunities to promote nutrient mineralization, allocation and availability via their symbiotic associations with plant roots. Many studies found that soil microorganisms influence the physical, chemical and biological properties of soil either directly or indirectly (summarized in Gyaneshwar et al. 2002; Jacoby et al. 2017; Saharan and Nehra, 2011 and Wang et al. 2017).

Despite tea soil microbiology being explored for centuries, knowledge of the tea soil microflora roles in enhancing tea cultivation is still poorly understood (Dutta and Misra 2010). The natural abundance of microorganisms within the tea agroecosystem is expected to play a significant role in maintaining a sustainable environment (Singh et al. 2011). Nepolean et al. (2012) examined the inoculation potential of soil microbes such as *Azotobacter*, *Azospirillum* and *Pseudomonas* in the tea plantations in Northeast India. A similar study using plant growth-promoting rhizobacteria (PGPR), such as *Serratia marcescens*, *Bacillus pumilus* and *Bacillus amyloliquefaciens* for the overall improvement of growth and productivity of tea was carried out by Chakraborty et al. (2013). Positive plant growth-promoting (PGP) traits, such as phosphate solubilization, siderophore production, antagonism to pathogens and indole acetic acid (IAA) production, were exhibited by these beneficial microorganisms, which successfully enhanced the seedling growth of tea varieties in the nursery as well as in the field. Due to the abundant inoculation of native antagonistic microbes, such as entomopathogenic viruses, bacteria, actinomycetes and fungi, they have also been recognized as alternative biocontrol agents against a wide variety of tea pests and pathogens, which has led to their introduction as an attractive component in integrated pest and disease management programs employed in tea plantations (Kodomari 1993). Moreover, the exploitation of diverse microbes such as PGPR and many other useful microorganisms might lead to improved nutrient uptake, plant growth and plant tolerance to biotic and abiotic stresses, which then can benefit tea productivity and efficiency.

1.7 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) could be found in all ecosystems and play a vital role in plant nutrition (Cavagnaro et al. 2015; Lee et al. 2013) and an important indicator of ecosystem health (Balsler et al. 2010; Jeffries et al. 2003). The importance of AMF has been widely recognized as

their roles in interacting with soil physical, chemical, and biological properties (Herrmann et al. 2016). Successful uses of AMF to achieve crop yield and nutrition improvements were widely reported from the wide range of different soil and cropping systems (Adholeya et al. 2005; Balliu et al. 2015; Borkowska et al. 2002; Emam, 2016; Xun et al. 2015). By contrast, very low levels of mycorrhization, which resulted in limited or even no impacts on targeting plants were also recorded as the consequences of inoculation with unidentified commercial products (Berruti et al. 2013; Faye et al. 2013; Tarbell and Koske, 2007). Additionally, there have been a variety of factors that affect the inoculation success, ranging from soil characteristics (Carrenho et al. 2007), crop compatibility (An et al. 2010; Gaur and Adholeya, 2002), the effectiveness and abundance of indigenous AMF composition and the nature of inoculants (Köhl et al. 2016; Verbruggen et al. 2013) to cultural practices (Palti 2012). These are governing factors that decide the AMF establishment as well as their consistency in soil for a few cultivation seasons (Köhl et al. 2016; Verbruggen et al. 2013).

Investigations into the relationship between tea trees and AMF have been poorly implemented. Presently, several studies have been conducted, but the results were inconsistent and seem to be site-specific. At field condition, Aliasgharzad et al. (2011) found no evidence for AMF association in tea roots in Iran whilst a study conducted in India (Singh et al. 2008) recorded a very high proportion of root colonization of both natural and cultivated tea in the year around (77.6% and 86.4% for natural and cultivated tea respectively during active growing seasons and 97% and 98 % respectively during the dormancy period). Similarly, Toman and Jha (2011) indicated that all tea root segments were colonized, and 20 AMF species were found associated with tea roots. In salty soil, it was reported that tea root colonization reduced as salt stress increased, which also resulted in the significant decline of tea yield (Liu et al. 2014). Other research was also carried out in this area however, they were either conducted in the nursery condition (Kahneh et al. 2006) or focused on the effects of inoculation with AMF on tea cultivation (Shao et al. 2018; Singh et al. 2010). In Vietnam and many

other tropical countries, tea trees have been planted in poor soil conditions. Since AMF could beneficially contribute to improve the nutrient status of the tree, thus resulting in improved tea productivity, the indigenous AMF structure and composition associated with tea plantations is a topic that deserves more attention. However, there has been very limited research implemented in Vietnam to examine the AMF- plants association (Sasvári et al. 2012) and to date, no study has been conducted to evaluate AMF- tea trees interaction.

1.8 Challenges and how to encourage agroecological tea management strategy

Despite rapid growth over the last few years, agroecological tea farming still accounts for only a small percentage of tea plantations in Vietnam. First, poor technical assistance, unavailability of inputs required for agroecological practices such as biofertilizers, biopesticides or organic fertilizer, small scale production and lack of understanding of long-term benefits of agroecological tea farming have been identified as the main limiting factors (Doanh et al. 2018; Tuan 2019; Van Ho et al. 2019). Furthermore, limited market information and linkages, especially that involve international markets such as legislation and standards regarding food safety and quality has been a great barrier with a majority of Vietnamese tea stakeholders (Ha 2014a; Thang and Hoa 2015). Moreover, the third-party certification processes of organic and VietGAP tea products are costly and time-consuming, preventing conventional tea farmers from adopting these cultivation methods (Ha 2014a; Van Ho et al. 2019). Finally, difficulty in accessing affordable credit for enhancing technology adoption and investing in the establishment period of agroecological tea production is also influencing tea producer decisions, especially in low-income small households (Doanh et al. 2018).

To promote agroecological tea farming, central and provincial governments must provide both technical and non-technical supporting policies and programs. For the technical aspects, the

governments and other relevant stakeholders should provide better extension services and organize relevant training to farmers, including on-farm trials to allow farmers to learn from each other and understand the benefits of this management implementation (Doanh et al. 2018; Tuan 2019). Furthermore, encouraging the commercialization of agrochemical alternatives would make these organic or biological inputs easily accessible to tea producers, even in remote areas (Ha 2014a; Van Ho et al. 2019). Improving market information and access to affordable credit are important priorities to support low-income tea farmers and encourage them to change their management practices towards agroecological managed fields. As a result, tea exporters would also invest more in high technology and advanced innovations to improve tea quality and productivity such as branding, post harvesting and marketing (Doanh et al. 2018 Thang and Hoa, 2015).

1.9 Thesis aims and chapter relevance.

Despite being a crucial cash crop and having a consistent growth of production volume since the 1990s, long term conventional tea farming in Vietnam has been facing many problems, including soil degradation and erosion, low tea quality and productivity and increased human health and environmental pollution concerns. In finding a more sustainable cultivation system to alleviate these challenges, a great number of Vietnamese tea stakeholders have been transitioning from this management method to agroecological tea management practices like using organic fertilizers and biofertilizers, mulching and intercropping as well as IPM and IDM. Numerous studies have indicated the beneficial effects of agroecological management practices such as using organic fertilizers (Li et al. 2015; Lin et al. 2019; Senapati et al. 2002), biofertilizers (Nepolean et al. 2012; Roychowdhury et al. 2014; Xu et al. 2014), biopesticides (Nakai 2014; Roychowdhury et al. 2014), mulching, intercropping (Jianlong et al. 2008; Sun et al. 2011; Zhang et al. 2017), IPM and IDM (Mamun and Ahmed 2011; Shrestha and Thapa 2015) and organic farming method (Wang et al.

2016b). These practices can result in soil health improvement (soil physical, chemical and biological properties); reduce agrochemical input costs, chemical residues in soil, tea leaves and mitigate the negative effects of chemical uses on the environment while maintaining tea productivity and quality. In Vietnam, the benefits of agroecological tea management, assessing profitability (Doanh et al. 2018; Duc and Goto 2019; Van Ho et al. 2019) and social and policy aspects (Ha 2014a) have been investigated to a limited degree. Studies also examine the impacts of mulching and biofertilizers on soil health (Cu and Thu 2014a, b) but to our knowledge, there has not been any study investigating the effects of other agroecological practices such as organic fertilizers, intercropping, non-pesticide pest and disease control methods. In addition, since organic, VietGAP farming and other agroecological cultivation approaches promote the application of multiple practices in a system (Hoang 2018; Vietnam Farmer's Union 2018), it is more significant to consider impacts of combined applications. Also, how these cultivation methods affect tea quality and productivity has not been determined, limiting the application and promotion of agroecological tea management to tea farmers and relevant organizations in Vietnam. Finally, as tea soil plantations in the studied region have been found to be strongly acidic, suitable options to deal with the issue such as liming have not yet been investigated.

The aims of this research are to provide a comprehensive overview of tea production in Vietnam, the challenges of conventional tea farming and potential benefits of agroecological tea management approach (chapter 1). Mechanisms and consequences of soil acidification by tea cultivation and potential uses of agricultural wastes to mitigate the issue as well as to enhance soil health and impacts of different tea management systems on soil physicochemical properties, colonization of AMF, tea productivity and production economic efficiency will be discussed in chapter 2. In addition, effects of lime application as a soil acidification management strategy and difference of land use history on tea soil physicochemical properties, soil and mulch macrofauna communities;

and diversity and composition of soil microbial communities and tea yield and yield components will be evaluated in chapter 3. The outcomes of this project will be an informative resource for tea producers, tea production management authorities and other relevant organizations in enabling more informed decisions regarding the management methods, policies and programs to promote agroecological tea management in Vietnam and other tea producing countries which share the similar natural and social-economic conditions. In addition, since organic and agroecological farming adaptation have been rapidly increased in Vietnam over the last decade, the strategies developed in this study might also be useful in understanding and improving the sustainable management of other perennial crops in the nation.

2. CHAPTER 2: Tea soil acidification and sustainable green tea production through agroecological management and land conversion practices for restoring soil health, crop productivity and economic efficiency: Evidence from Northern Vietnam

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See the “Authorship statement – Chapter 2” (appendix 2 and appendix 3) for details.

2.1 Abstract

Tea is one of the world's most consumed beverages and an important crop of many developing countries. As tea plants can retain their productive life span for decades, intensive tea cultivation has negative impacts on soil health properties and the environment. In Vietnam, tea is a particularly important cash crop as it supplies crucial income and employment for farmers in poor rural areas. Unfortunately, the dominance of long-term, conventional tea cultivation has caused severe soil health degradation and environmental pollution. At the same time, as tea production may provide a better net income compared to other annual crops such as rice and vegetables, farmers have been converting parts of their allocated lands to cultivate tea plants.

While soil acidification in tea plantations is a globally known severe issue, there is a lack of literature analysis of the ways in which soil acidification affects soil health, tea productivity and the environment, and suitable methods to control this issue. Additionally, little is known about the benefit of agroecological management as an alternative to conventional tea management practices and thus there is a need to understand how it can improve tea yields, quality and the livelihoods of the farmers. Here, we review the mechanisms of tea soil acidification and consequences; the potential of common agricultural wastes for ameliorating soil acidity and enhancing soil health and crop productivity, as well as reducing environmental pollution under tea cultivation. We also conducted a field study in Northern Vietnam from 2019-2022 to examine the impacts of agroecological tea management practices on soil health indicators, tea yield and quality, and net income of tea farmers.

We show that intensive application of mineral nitrogen is the main cause of soil acidification in tea plantations, while tea plants also play a part in accelerating tea soil acidity. Agricultural waste and by-products have a great potential to correct soil acidity, enhance soil health and tea productivity

and quality. These soil amendments also have drawbacks such as heavy metal and pathogen pollution, and supplementary costs that require consideration. Furthermore, agroecological management practices significantly enhanced soil organic matter by 0.8% and soil pH by 0.5 units on average, compared with the conventional management approach. Conversely, conventional management based on chemical fertilizer applications significantly increased soil total nitrogen by 0.15-0.2%. No significant differences were observed between soil texture and other soil chemical characteristics. Soil biological parameters were also significantly higher in agroecological tea soil and root samples than in conventional tea plots. Average AMF frequency and intensity of the agroecological tea roots were 98% and 37%, respectively, compared with 73% and 15% of the conventional tea roots. Likewise, soil macrofauna and mesofauna abundance in the agroecological tea plantations was 76 individuals/m² and 101 individuals/100 g fresh soil on average, respectively, while that of conventional tea farms were 34 and 63 individuals/100 g fresh soil, respectively. Interestingly, comparison between the converted and non-converted lands did not show any significant effect of the conversion on soil physicochemical and biological characteristics, apart from tea root AMF colonization. Conventional tea management consistently resulted in higher tea yield and yield components, even though the differences were not always statistically significant. Despite lower tea yields, agroecological tea adopters earned around USD 8,400 ha/year more than the farmers still practicing conventional management. This study shows that it is economically and environmentally more sustainable to produce organic tea than conventional tea and our results should encourage more farmers to adopt such agroecological practices in Northern Vietnam.

2.2 Introduction

Soil acidification has been a major threat to soil health and environmental sustainability in various agricultural systems and regions (Dai et al. 2017; Li et al. 2016a; Yan et al. 2020) and occurs in many tea growing countries, such as China (Lin et al. 2019; Ni et al. 2018; Zou et al. 2014), India (Bandyopadhyay et al. 2014), Japan (Oh et al. 2006), Sri Lanka, Rwanda (Mupenzi et al. 2011), and Vietnam (Huu Chien et al. 2019). In China, the leading global tea producer and exporter, greater soil acidification occurred in tea plantations compared to other cash and cereal cropping systems, with 46% of tea plantations nationwide reporting soil pH below 4.5 (Yan et al. 2020). The reduction of soil pH in tea plantations will have impacts of soil characteristics by changing soil chemical processes, resulting in soil nutrient losses and imbalance, and increasing occurrence of Al and Mn toxicity (Alekseeva et al. 2011; Ni et al. 2018; Yan et al. 2018). In addition, soil acidification significantly degrades the diversity and functionality of soil organisms (Goswami et al. 2017; Li et al. 2017). While soil acidification occurs naturally in tea plantations and increases with increasing tea plant age and plant density, intensive application of mineral nitrogen (N) is the main cause of the issue (Li et al. 2016a; Yan et al. 2018).

Tea (*Camellia sinensis* Kotze) has been cultivated for centuries and plays an important role in economic development and social sustainability in Vietnam (Bui and Nguyen 2020; Viet San et al. 2021). Currently, tea plantations cover an area of around 130,000 ha, with over 1 million tons of fresh tea leaves being produced annually (Viet San et al. 2021). Since 2010, Vietnam has been among the top five leading tea exporters worldwide, with the annual revenue from tea exports over USD 200 million per annum (Van Ho et al. 2019). In Vietnam, tea is mainly grown in the Northern mountainous areas, where conventional management method has been the dominant practice (Doanh et al. 2018; Viet San et al. 2021). Long- term intensive application of agrochemicals under

conventional tea cultivation in this region has resulted in a range of serious issues, such as soil health and environmental degradation, human health concerns and reduced tea quality (Van Ho et al., 2019; Viet San et al., 2021). Recently, Vietnam has experienced an increasing transition from conventional tea cultivation to other alternatives such as organic and agroecological tea management practices (Ha 2014; Van Ho et al. 2019). Apart from existing conventional tea areas, tea growers also convert their allocated croplands such as paddy rice and vegetable fields to cultivate tea crops. These conversions have been driven by the growing interests in greater economic efficiency of tea production compared to other annual crops, high tea quality as well as an increased awareness of agrochemical detrimental effects on human health and the environment (Doanh et al., 2018; Viet San et al., 2021).

Soil health can be defined as the capacity of a soil to provide ecosystem services and it has been typically assessed by considering all the attributes including soil physical, chemical and biological properties (Ippolito et al. 2021; Williams et al. 2020). Different agricultural management practices can lead to long- term and differing effects on soil health properties (Bai et al. 2018). For instance, conventional agriculture which employs intensive agrochemical inputs has been widely known to negatively impact soil health in comparison with conservation and organic farming (Singh et al. 2020; Viet San et al. 2021). In contrast, the role of agroecology in restoring soil health, providing sustainable food production and environmental benefits has been increasingly recognized worldwide (Dumont et al. 2021; FAO 2020; Nicholls and Altieri 2018). Agroecological practices aim at optimizing agroecological processes, environmental and public health whilst minimizing social-ecological costs from agricultural activities (FAO 2020; Kerr et al. 2021). For tea farming, numerous studies outside Vietnam have indicated the positive impacts of agroecological practices on soil health properties and tea quality indicators, such as the application of organic fertilizers (Gu et al. 2019; Han et al. 2021; Lin et al. 2019) and organic mulching (Zhang et al., 2020). Similar

positive outcomes have also been recorded from other agroecological practices such as intercropping (Wen et al. 2019; Zhang et al. 2017), agroforestry (Tian et al. 2013) and integrated pest/disease management (Mamun and Ahmed 2011; Shrestha and Thapa 2015). However, all these studies focused on impacts of agroecology tea management on soil microbial communities and their structures. Soil fauna and root mycorrhization with arbuscular mycorrhizal fungi (AMF) have been largely undocumented, while they play a key role in the decomposition of the organic matter and the mineral plant nutrition.

Land use history will also have significant and direct impacts on soil health due to subsequent alterations of management practices, vegetation cover and soil organism communities (Graham et al. 2021; Rasouli-Sadaghiani et al. 2018). Previous studies have consistently reported serious degradations of soil health as the consequences of converting forestlands and grasslands to croplands (Berkelmann et al. 2020; Gholoubi et al. 2018; Yang and Zhang 2014). Yet, how crop conversion affects soil health properties and which mechanisms are involved have received less attention and in the specific case of tea plantations, several studies showed the negative impacts of land conversion from forestlands or perennial croplands to tea cultivation (Gholoubi et al. 2018; Wu et al. 2020b; Zheng et al. 2020). These studies, however, did not focus on tea soil fauna communities, root AMF, as well as tea productivity, quality and the economic value of the conversion.

The use of agricultural organic waste products to ameliorate soil acidification has been recognized in agriculture systems worldwide (Cai et al. 2015; Cornelissen et al. 2018; Dai et al. 2017). By definition, agricultural wastes or agriculture by-products are the unwanted residues generated from agriculture activities, such as crop residues, animal manure, forest waste, vegetable matter and weeds (Dai et al. 2018; Ramírez-García et al. 2019). Animal wastes, green manures and products

derived from these wastes such as biochar and compost are generally alkaline in nature and have high pH buffering capacity which can neutralize soil acidification (Cai et al. 2021; Rayne and Aula 2020). Also, the presence of basic cations such as Mg^{2+} and Ca^{2+} , and organic anions in these materials contribute to increased soil pH (Cai et al. 2021; Tang et al. 2013). In addition to increasing soil pH, agricultural wastes have long been known to enhance soil health, including soil physical, chemical and biological properties (Bhatt et al. 2019; Cai et al. 2021; Rayne and Aula 2020). Globally, an estimated of 1 billion tons of agricultural wastes per year is generated, which China, USA and India being the largest agricultural waste producing nations worldwide (Fig. 1) (Clauser et al. 2021; Obi et al. 2016), and this figure has been projected to increase rapidly because of the growing demand of agricultural products (Dai et al. 2018; Wei et al. 2020b). Thus, the utilization of agricultural wastes as soil amendments could be a win-win strategy, which can benefit not only soil health but also reduce the pressure of using fossil fuels, mitigate serious environmental problems and human health threats (Bijarchiyan et al. 2020; Mpatani et al. 2021).

This chapter provides an overview of mechanisms and consequences of soil acidification by tea cultivation, the utilization of agricultural wastes and its products on mitigating soil acidification and enhancing soil health properties under tea plantations. By conducting a comprehensive field assessment in four communes of the Thai Nguyen province, we also investigated how different management practices and land use history affect soil physical, chemical and biological properties, tea productivity, quality and economic efficiency of tea production in the region. The outcomes of this study develop an understanding of the mechanisms and consequences of soil acidification by tea cultivation, the role of soil physicochemical properties, root arbuscular mycorrhizal fungi and soil fauna communities in maintaining soil health and tea productivity and quality in the Acrisols soils in Thai Nguyen province as well in Northern region of Vietnam, and the sustainability of

agroecological tea management practices in the region in comparison with the conventional approach.

2.3 Soil acidification by tea cultivation and its consequences

2.3.1 Ocean and soil acidification

Ocean and soil acidification have been widely reported as the most critical issues, affecting the sustainability of numerous ecosystems and regions around the world (Ochedi et al. 2021; Yan et al. 2020). Ocean acidity has increased by ~25% since the 1860s, and the soil pH values of 50% of total arable land worldwide are below 5.5 (Dai et al. 2017; Hall et al. 2020). Ocean acidification appears due to rising atmospheric carbon dioxide (CO₂) concentrations and absorption by seawater, which subsequently leads to a fall of pH and carbonate ion concentrations in surface seawater (Agostini et al. 2018; Sharma and Gunasekare 2018). Ocean takes up around 25% of global anthropogenic CO₂, making it the largest atmospheric CO₂ absorbent on Earth (Hauck (Hauck and Völker 2015). Among the CO₂ emission sources, agriculture directly contributes around 14% of the total amount globally, and this proportion is likely to be exceeded in the future (Ayyildiz and Erdal 2021). Intensive agriculture and land use practices have been also the main causes of global soil acidification, particularly inappropriate uses of ammonium-based fertilizers (Cai et al. 2015; Dai et al. 2017) Dai et al. 2017). Additionally, soil nutrient leaching, product removal, acidic parent materials, acid deposition and host plants are all likely to be significant factors resulting in soil pH reduction (Tang et al. 2013; Yan et al. 2020).

2.3.2 Soil acidification in tea plantations

Tea plant

Tea (*Camellia sinensis* Kotze) is one of the oldest and most popular beverages in the world, and is an important crop being cultivated in around 50 countries (Gebrewold 2018). Global tea production in

2019 was more than 9.2 million tons, valued at approximately \$US55.3 billion (Fig. 3) (Allied Market Research 2020; Food and Agriculture Organization (FAO) 2021).

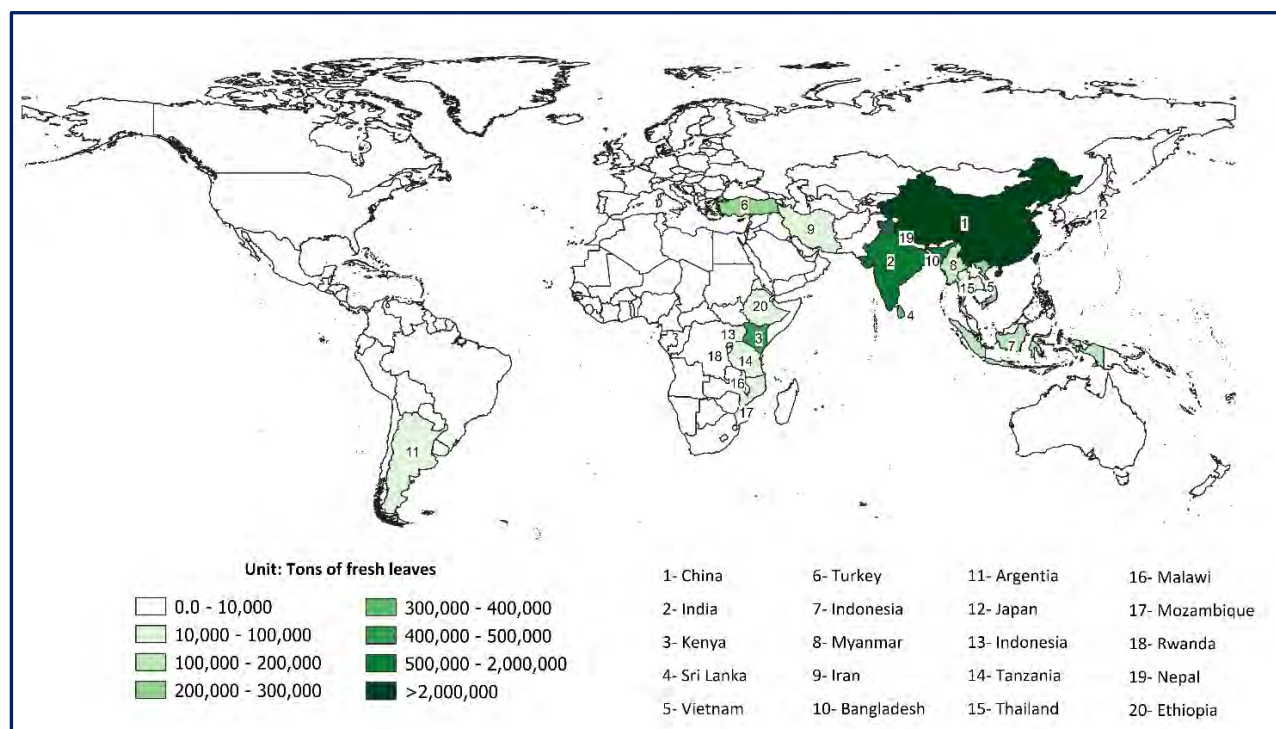


Figure 3. Map of the 20 world's largest tea producing nations in 2019. China was the largest tea producer worldwide in 2019, followed by India, Kenya, Sri Lanka and Vietnam. Most of the global tea producers are in Asia and Africa. The top 20 global tea producing countries contributed around 70% of total global tea production volume in the same year. Data was retrieved from FAO (2021)

Tea plants are native to the Asian continent, but they can adapt to a wide range of soil and climatic conditions (Rana et al. 2021; Yan et al. 2018; Yao et al. 2012). This perennial crop requires acidic soils for optimum growth and productivity, with the optimal soil pH for tea plants being between 4.5- 6, and the plants themselves are capable of acidifying soil (Fig. 4) (Gebrewold 2018; Li et al. 2016). Being a woody perennial, tea plants can maintain their productivity for decades, and thus have long-term interactions with soil organisms and physicochemical processes, affecting soil health and plant productivity (Arafat et al. 2020; Yan et al. 2020).

Soil acidity by tea cultivation practices

Soil acidification in tea plantations results predominantly from inappropriate management practices, particularly the intensive overuse of mineral N (Li et al. 2016a; Yan et al. 2018). Tea growers apply N to ensure high tea productivity and as a replacement for soil nutrient loss. In Japan, tea fields are amended with more than 1000 kg/ha of N fertilizers per annum (Abe et al. 2006; Zou et al. 2014) and a majority of tea farmers in China apply a large amount of N to ensure high tea yield and maintain soil fertility (Yan et al. 2018). A recent study has shown that nitrogen fertilizer application rate can even reach 1200 kg/ha in Chinese tea plantations (Wu et al. 2016b). Soil pH significantly reduces when N fertilizers such as ammonium nitrate and urea are applied above 50kg/ha/year, and increased N addition rate will accelerate soil acidification (Tian and Niu 2015). Moreover, heavy N application results in greater decrease of subsoil pH compared with that of the topsoil (Ni et al. 2018). When fertilizers are applied at 2700 kg/ha, only 18,3% of applied nitrogen were absorbed by tea plants and, about 52% of nitrogen were stored in the soil, and 30% were lost through runoff, polluting surrounding watercourses and soils (Chen and Lin 2016; Xie et al. 2021).

The main mechanisms of soil acidification resulting from inappropriate management practices in tea cultivation are shown in Fig. 4. When NH_4^+ -N fertilizer is applied, tea plants directly take up the nutrient and tea roots subsequently excrete an equivalent proton into the rhizosphere, causing the concentration of hydrogen ions to increase. NH_4^+ nitrification leads to a net production of 2 mol H^+ for each mol of NH_4^+ applied, contributing to the decrease of soil pH (Hui et al. 2010; Li et al. 2016a; Yan et al. 2020). Cai et al. (2015) estimated that an application rate of 300kg/ha/year of N fertilizers could produce 21.4 kmol H^+ /ha/year by the nitrification processes. N fertilizer application in the long term also promoted the accumulation of exchangeable Al^{3+} including hydrolysis, which further generated H^+ and aggravated the acidification of tea plantation soils (Zhang et al. 2020). Finally, increasing tea plant age and planting density also result in an increase of organic and

carbonic acids induced by tea roots into the rhizosphere, which facilitate soil acidification (Hui et al. 2010). Tea plantation soil is not acidified at planting densities of 5000 plants/ ha (Li et al. 2016a).

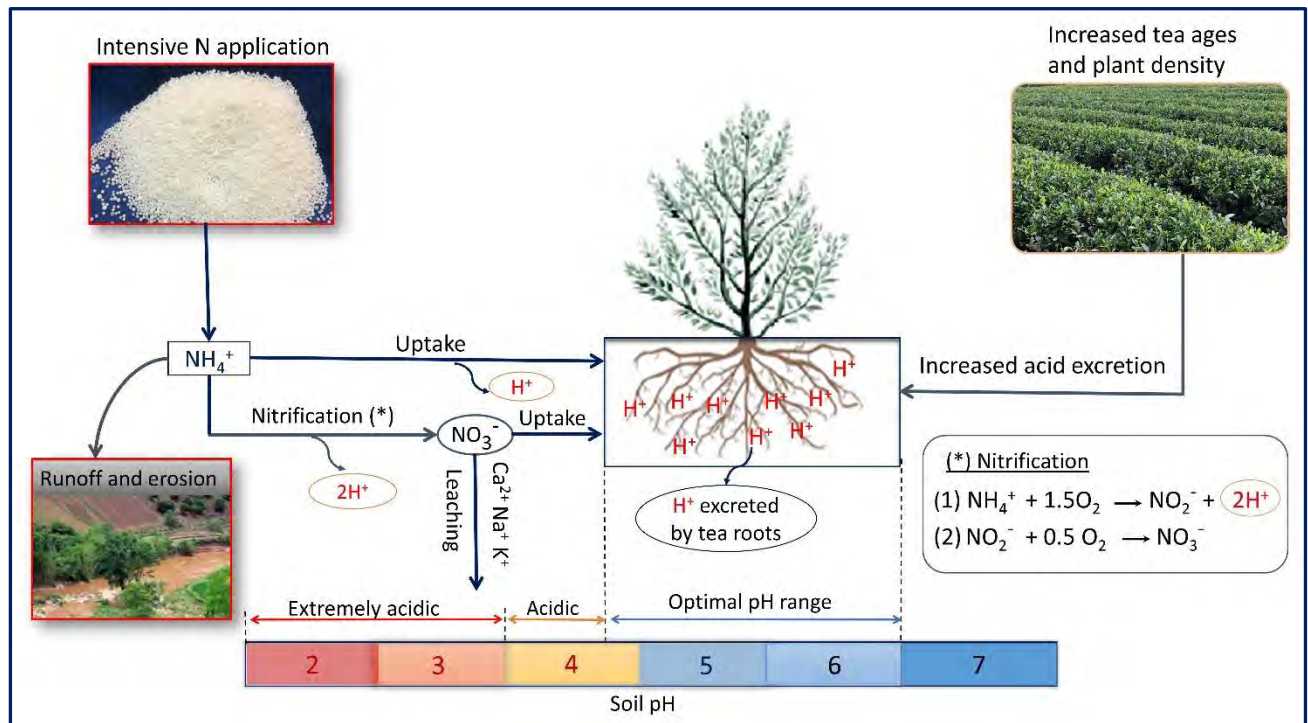


Figure 4. The main mechanisms of soil acidification by tea cultivation. Heavy addition of N fertilizers is the main reason causing soil acidification, and the accumulation of organic and carbonic acids released by tea roots also play a part in acidifying tea plantation soils

Soil acidification by tea plants

Acidification of soils may naturally occur in soils cultivated with tea – even without any imposed N proton additions, and this issue becomes more challenging with increasing tea plantations (Arafat et al. 2017; Han et al. 2007b; Li et al. 2016a). In tea plantations, soil pH in the topsoil naturally decreased by 0.071 units per annum, and the values following 13, 34 and 54 years of tea cultivation were 1,1; 1,62 and 2,07 units respectively (Hui et al. 2010; Ni et al. 2018). The acidification rate observed in the cultivated soil layers (0-10cm) could reach 4.40 kmol H^+ /ha/year during the 0-13 years of tea cultivation period (Hui et al. 2010). Organic acids secreted by tea roots such as malic

acid, citric acid, and oxalic acid are the main proton source for soil acidification in the tea tree- soil systems (Fig. 4) (Yan et al. 2018). Tea roots also excrete carbonic acids and polyphenols which can aggravate soil acidification, affect soil nutrient release and subsequent element uptake (Ni et al. 2018; Wang et al. 2013b). Additionally, the accumulation of chemical compounds such as epigallocatechin gallate, epigallocatechin, epicatechin gallate, catechin, and epicatechin, found in the tea residues also negatively affect soil pH and soil health properties (Arafat et al. 2020). In summary, intensive application of N fertilizers is the main cause of soil acidity under tea plantations, and the accumulation of acid excreted by tea plants promotes acidification.

2.4 Consequences of acidification in tea plantation soils

2.4.1 Soil chemical parameters

Soil acidification negatively affects chemical processes and properties of tea plantation soils (Fig. 5). One of the most serious challenges of soil acidification under tea cultivation can be the reduction and imbalance of nutrient base cations, including Ca^{2+} , Mg^{2+} , Na^+ and K^+ (Alekseeva et al. 2011; Ni et al. 2018; Zhang et al. 2020). Under heavy N application, released protons (H^+) may replace the soil exchange base cations, which may have leached with the NO_3^- as accompanied cations due to the charge balance in soil solutions (Cusack et al. 2016; Ni et al. 2018). Moreover, a significant increase of Al^{3+} and Mn^{2+} has been widely recorded in acidic tea plantation soils, which could lead to Al and Mn toxicity (Alekseeva et al. 2011; Hui et al. 2010). Under acidic soil conditions, mineral Al solubilizes into trivalent Al^{3+} , which is highly toxic to animals, plants and microorganisms (Zioła-Frankowska and Frankowski 2018). Gruba and Mulder (2015) indicated that the concentration of exchangeable Al maximizes in soils with a $\text{pH}_{\text{H}_2\text{O}} \approx 4.2$. Similarly, with decreasing soil pH, the amount of exchangeable Mn^{2+} increases in the soil solution (Millaleo et al. 2010). High concentration of Al^{3+} can inhibit the expansion, elongation, and division of root cells, reducing

water and nutrient uptake by the root systems (Wang et al. 2015). Similarly, high levels of Mn^{2+} in soil is one of the main factors causing nutrient imbalances, especially with divalent cations such as Mg^{2+} , Zn^{2+} and Ca^{2+} (Venkatesan et al. 2010). Soil acidification can also promote the dissolution of minerals and movement of Fe in the profile, resulting in reduction of ferrimagnetic mineral content (Alekseeva et al. 2011). Increased Al and Mn toxicity have been considered as the most serious consequences of soil acidification by tea cultivation regarding soil chemical property.

2.4.2 Soil biological parameters

Soil pH is a crucial factor affecting soil organisms (Li et al. 2018; Neina 2019). Mulder et al. (2005) indicated that soil acidification has a close inverse relationship with bacterial, fungal, nematode and arthropod abundance. Long-term soil acidification is responsible for reduction of soil microorganisms, which are regulating the reduction in soil pH by both ecological and evolutionary mechanisms because of the environmental changes (Zhang et al. 2015). For instance, soil fauna communities were significantly higher in the soil with pH 7.0 (21 classes) compared to acidic soil with pH 2.5 (11 classes) and pH 3.5 (14 classes). In the study, in terms of total individuals, the figures were 3710 (pH 7.0); 759 (pH 3.5) and 645 (pH 2.5) (Wei et al. 2017). Severe soil acidification also leads to significant decreases in soil enzymatic activities, microbial activities, and microbial biomass (Li et al. 2017; Zhang et al. 2015). Arafat et al. (2019) found a close association between the decline of some beneficial fungus such as *Mortierella elongatula* and *Mortierella alpina* and a low soil pH caused by long-term tea monoculture. Soil acidification also enhances the environment for growth of some soil-borne pathogen diseases. For instance, when soil pH reduced from 5.07 to below 3.5 as a result of 35 years of continuous tea monoculture, the abundance of some pathogenic bacterial species including *Fusarium oxysporum*, *Fusarium solani*, and *Microdidium phyllanthi*, which are responsible for diseases in tea plants such as root rot and die back, was significantly increased (Arafat et al. 2019). Investigating the relationship between soil acidity and

bacterial wilt disease, Li et al. (2017) found that the proportion of soil affected by bacterial wilt is much higher when the soil pH is lower than 5.5, and significantly less as the soil pH increases. Likewise, the highest population of *Xiphinema chambersi* was found in soil with a pH 4.5, and the figure decreased when soil pH increased from 4.5 to 6.4 (Chen et al. 2012). Thus, soil acidification by tea cultivation could not only impact soil beneficial microbial diversity, but also promote the development of some potentially pathogenic microbes (Fig. 5).

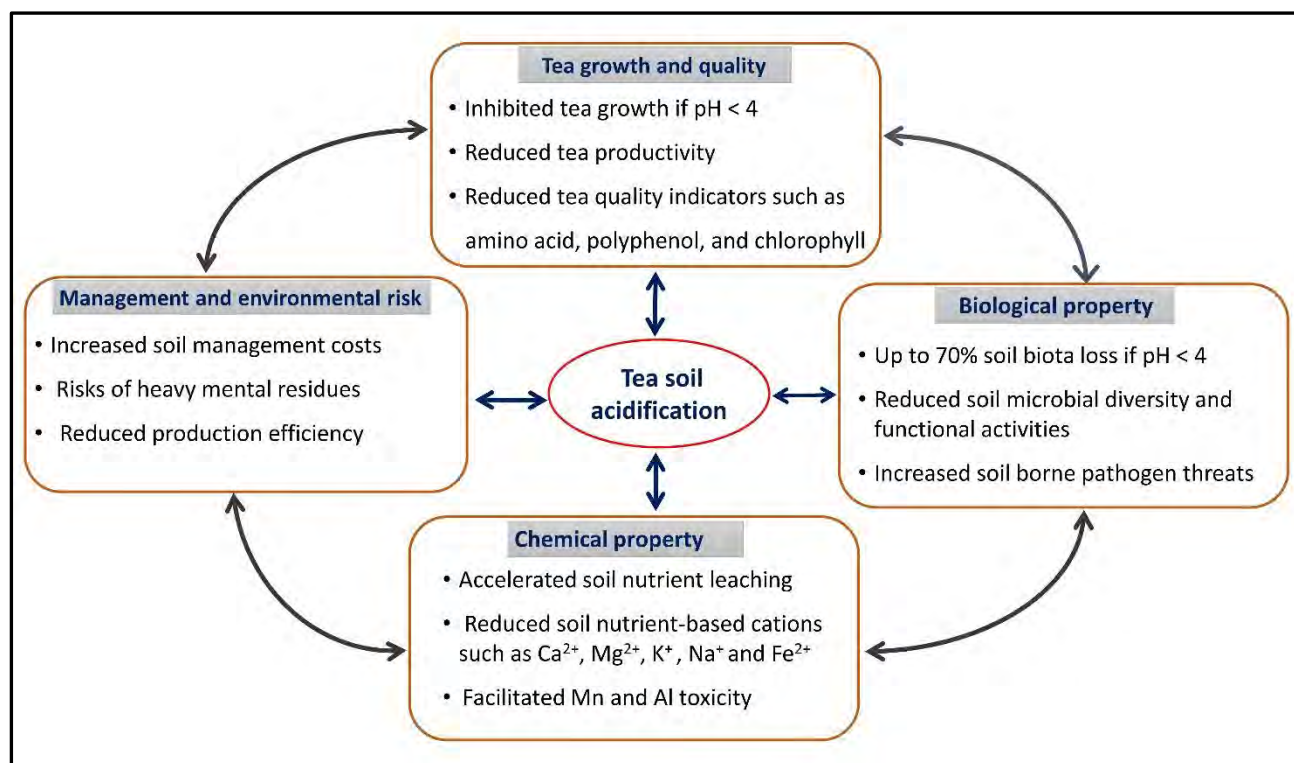


Figure 5. A summary of the main consequences of soil acidification caused by tea cultivation in the aspects of soil chemical and biological properties, tea growth and quality, soil management cost and the environmental risks

2.4.3 Tea productivity and quality

Although tea plants prefer acidic soil for optimal growth and productivity, severe soil acidity negatively affects plant performance and quality (Fig. 5). Based on both field and controlled

experimental studies, it was reported that when the soil pH is lower than 4.0, tea plant growth is inhibited, affecting both the quality and quantity of tea production (Li et al. 2016; Yan et al. 2020). Heavy N addition also significantly decreases the Polyphenol/free amino acid ratio and affects other tea quality indicators by altering the relative content of chemical constituents (Qiao et al. 2018). High concentrations of Mn^{2+} negatively affect tea quality indicators such as amino acid composition and reduce the chlorophyll and carotenoid content of tea leaves (Venkatesan et al. 2010). Free Al^{3+} at a concentration of more than 1 mM retards tea growth, while the concentration at 10 mM leads to defoliation of tea plants (Fung et al. 2008).

2.4.4 Management cost and environmental risks

Despite the limited study on the management and other associated costs of soil acidification in the tea farming industry, various studies highlight negative impacts of soil acidification on other agricultural sectors. For instance, the annual loss of agricultural production due to soil acidification in New South Wales, Australia was around \$387 million (Li 2020). Likewise, soil acidification resulted in an estimated economic value decrease of \$US214,000 per hectare (ha) in the forest industry in America (Caputo et al. 2016). Lime has been considered as the most effective ameliorant to control acidic soils, but it is still too costly for farmers in many countries, due mainly to its transportation costs (Cai et al. 2015; Tang et al. 2013). In tea plantation soils, acidification also occurs at the subsoil layers (100-120cm), thus deep incorporation of lime and other alternatives could be very expensive or even impractical due to the costs of suitable machinery (Li et al. 2016; Tang et al. 2013). Tea soil acidification can also promote the accumulation of chemical elements such as arsenic (As), mercury (Hg), lead (Pb), chromium (Cr), cadmium (Cd) and nickel (Ni) in the soil and tea leaves, increasing the human health and environmental risks of heavy metals (Bayraklı and Dengiz 2020; Zhang et al. 2020). It has been reported that more than 75% of soil Cd, Hg, Pb and Zn under acidic tea plantations exceeded uncultivated background concentrations, possibly due

to the acidic environment promoting weathering pedogenic process releasing heavy metals (Tao et al. 2021).

2.5 Agricultural wastes for correcting tea soil acidification and enhancing soil health

2.5.1 Agricultural wastes for soil acidification and soil health

Agricultural wastes such as organic manures have been considered as a significant resource for agriculture for over hundred years (Rayne and Aula 2020), and since the downsides of agrochemical intensification on human beings and the ecosystem have become a global issue, the potential role of these alternate materials is being scrutinised increasingly closely (Chen et al. 2018; De Corato 2020). Most agricultural wastes are widely available, cheap, biodegradable and rich in organic matter and nutrients and thus can be recycled as fertilizers or soil amendments (Kaur 2020; Onwosi et al. 2017; Saliu and Oladoja 2021). The nutrient compositions of agricultural wastes and products derived from these resources varies greatly and depend on multiple factors, such as their original sources, animal diets, waste storage and management, as well as production procedures (Amoah-Antwi et al. 2020; Dai et al. 2017; Rayne and Aula 2020). Common agricultural by-product and their components applied to agricultural soils as fertilizers and amendments are illustrated in Fig. 6.

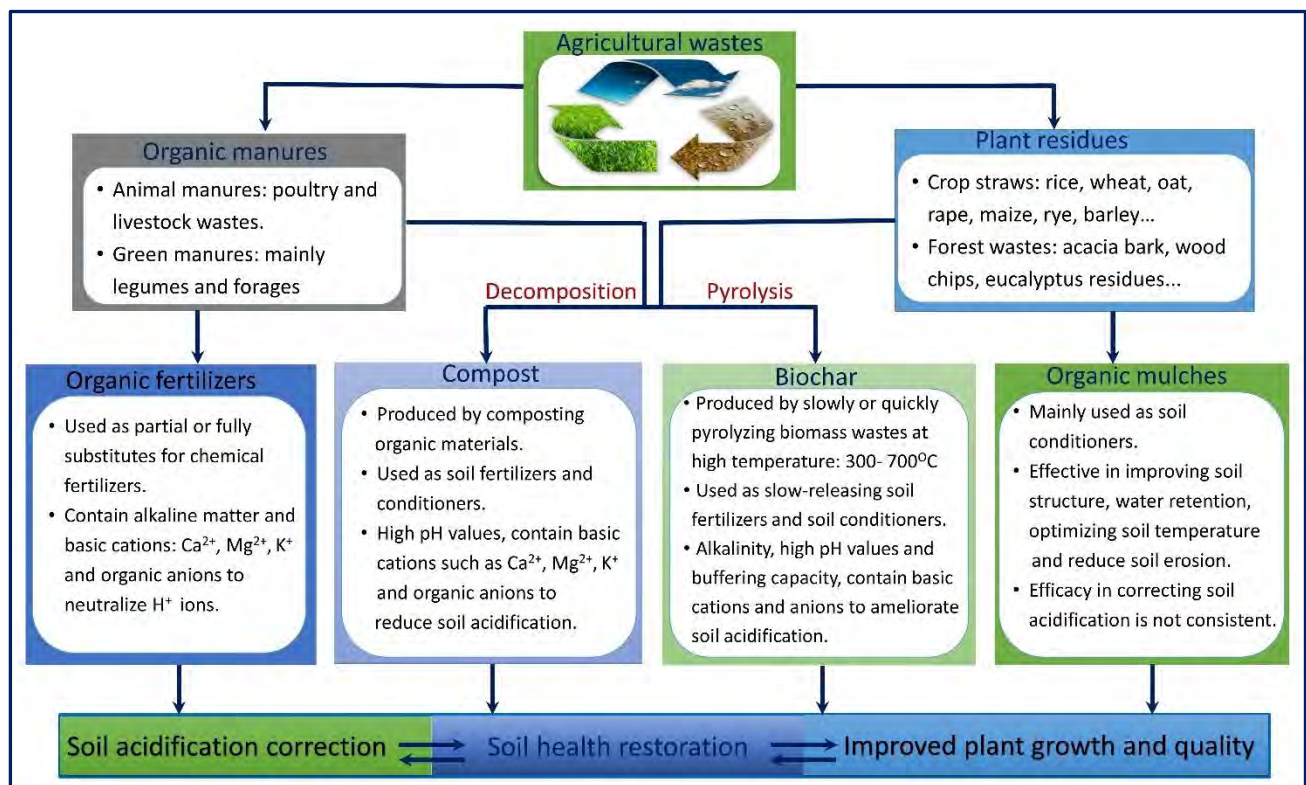


Figure 6. Common types of agricultural wastes and products using these wastes as main feedstocks, how they could be produced and used to mitigate soil acidification and improve soil health, crop growth and quality

There are various types of agricultural organic wastes applied to croplands, but they can be divided into two different groups based on their origins and common uses (Fig. 6). Organic manure includes animal wastes from livestock and poultry industries, and green manures are mainly leguminous and forage crops (Maitra et al. 2018; Rayne and Aula 2020). Globally, animal waste has been predominantly attributed to manure from livestock and in 2018, contributed around 35 million tons of N applied to croplands globally, compared to more than 13 million tons from poultry (FAO 2021). Organic manures can be applied to soils or used as main materials for compost production, the natural biological processes of decomposing organic wastes involving numerous microbial species (Azim et al. 2018; Bhatt et al. 2019; Sánchez et al. 2015). Compared to manures and compost, plant straws and other organic biomass such as wood chips and tree pruning residues are

not often applied directly to soils as fertilizers, but can also be incorporated as mulches, mainly for enhancing soil structure and water retention (Amoah-Antwi et al. 2020; Siedt et al. 2020). Alternatively, using agricultural by-products to produce biochar has also been an increasingly accepted way of recycling wastes. Biochar could be best described as a “soil conditioner”, a rich carbon product produced by thermochemical decomposition of organic matter under low oxygen environment and high temperature, normally from 300- 700⁰C (Peng et al. 2018; Verheijen et al. 2010). Feedstocks for biochar production consist of various biomass types, including municipal wastes and agro-industrial residues, and the feedstock types are important factors affecting biochar properties (Amoah-Antwi et al. 2020; Gunarathne et al. 2019; Guo et al. 2020). Details of elemental properties of some common agricultural wastes, compost and biochar are summarised in Table 3.

The various agricultural wastes have differing effects on alleviating soil acidification. Organic compost and biochar produced from organic manures and plant residues are naturally alkaline and have a higher pH value compared to that in the acid soils, so the addition of these organic amendments can increase soil pH to some extent (Cornelissen et al. 2018; Shi et al. 2019). Additionally, organic manure and its components naturally contain some basic cations such as Mg²⁺, Ca²⁺, Na²⁺ and K⁺, which can form carbonates or oxides and then subsequently react with the H⁺ in the acidic soils and lead to the acid neutralization (Dai et al. 2017; Rayne and Aula 2020). In contrast, some studies showed that the decomposition of some mulching materials such as woody chips, crop straw and pine bark could generate organic and carbonic acids, which facilitate soil acidity (Arafat et al. 2020; Zhao et al. 2018). Nevertheless, numerous studies have reported the neutral to positive effects of mulching practices on soil acidification (Ni et al. 2016; Sadek et al. 2019; Vijay 2014).

With regards to soil physical aspects, plant residues, organic fertilizers and biochar applications can

benefit soil hydrothermal environment, soil structure and water holding capacity (Kader et al. 2017; Siedt et al. 2020; Wang et al. 2020). In terms of soil chemical properties, adding organic fertilizers and biochar significantly improve soil organic matter, soil macronutrients and micronutrients, reduce Al and Mn toxicity risks and nutrient leaching (Ding et al. 2020; Gong et al. 2020; Patra et al. 2021; Siedt et al. 2020; Zhongqi et al. 2016). Recently, a number of studies have reported the positive impacts of agricultural residue practices on soil organism abundance and functional diversity, such as the applications of organic mulches (Xiang et al. 2021; Zhang et al. 2020b), biochar and compost (Amoah-Antwi et al. 2020; Liu et al. 2021) and organic manures (Rayne and Aula 2020; Su et al. 2021). Despite the preference in using synthetic fertilizers, agricultural wastes and products derived from these resources are being used intensively as soil amendments and fertilizers, to partially or fully substitute for chemical fertilizers (Amoah-Antwi et al. 2020; Lin et al. 2019; Shaji et al. 2021). However, since the nutrient compositions and efficacy of agricultural wastes and its products varied significantly (Table 3), they cannot be applied in a homogenous manner (Dai et al. 2017; Rayne and Aula 2020). Therefore, having a good understanding of characteristics of agricultural wastes and its components would be important to increase their application efficiency and reduce the pollutant risks to ecosystems (Amoah-Antwi et al. 2020; Ayilara et al. 2020; Cai et al. 2021).

Table 3. Nutrient composition of some main types of agricultural wastes and its based products used as soil amendments in tea cultivation and croplands

Type of waste	Nutrient composition									Reference	
	N	P	K	Na	Fe	Cu	Mn	Zn	Total C		
1. Animal manure											
Horse	20.7	7.6	41.4	7.58	729	22	110	167	43.3	Moreno-Caselles et al. (2002); Chong et al. (2019)	
Cow	18.6	7.89	17.6	5.38	3527	20	111	79	43.88	Mendonça Costa et al. (2015); Moreno-Caselles et al. (2002)	
Calf	17.5	9.6	35.1	24.6	2839	40	225	233	-	Moreno-Caselles et al. (2002)	
Pig	21.7	14.4	8.9	2.34	1559	170	328	427	-	Moreno-Caselles et al. (2002)	
Sheep	18.7	5.67	34.3	6.94	3786	21	137	159	41.84	<i>Mendonça Costa et al. (2015);</i> <i>Moreno-Caselles et al. (2002)</i>	
Goat	22.2	8.1	59.2	16.9	1729	31	170	202	-	Moreno-Caselles et al. (2002)	
Rabbit	17.9	9.2	18.2	5.07	2623	61	225	453	-	Moreno-Caselles et al. (2002)	
Chicken	31.4	13.2	24.7	4.85	154	40	237	304	34	Moreno-Caselles et al. (2002); Ravindran and Mnkeni (2016)	
Turkey	39.7	10.9	24.5	3.97	172	45	327	336	39.7	Moreno-Caselles et al. (2002); Calbrix et al. (2007)	
Ostrich	16.5	7.7	10.7	4.64	1303	56	257	200	-	Moreno-Caselles et al. (2002)	
Earthworm	17.3	11.9	7.8	2.34	6503	78	335	348	-	Moreno-Caselles et al. (2002)	
Note: N, P, K (g/kg, dry weight); Na, Fe, Cu, Mn, Zn (mg/kg, dry matter); Total C (%), dry weight).											
2. Plant residues	N	P	K	C	Ca	Mg	pH	C:N ratio	Ash content		
Wheat straw	55	9	42	43.9	22.61	2.88	5.1	124.4	23.2	Torma et al. (2018), Zhao et al. (2018), Plazonić et al. (2016)	
Potatoes	59	6	61	-	-	-	6.1	22.0	20.4		
Maize straw	39	3	19	42.14	6.40	4.60	-	-	48.8		
Oat straw	55	8	58	36.35	-	-	-	54.25		Torma et al. (2018); Zhao et al. (2018)	
Rye	45	8	24	-	-	-	-	-	-	Torma et al. (2018); Wang et al. (2009)	

Barley	43	7	40	-	-	-	-	-	7.14	Torma et al. (2018); Plazonić et al. (2016)
Triticale	54	8	28	-	-	-	-	-	5.27	
Pea straw	112	14	74	43.56	17.32	6.51	-	-	61.6	Wang et al. (2009)
Soybean straw	132	14	72	44.06	18.24	17.86	-	44.06	72.0	Jalali and Ranjbar (2009); Torma et al. (2018)
Sugar beet	20	2	13	-	-	-	-	-	-	Torma et al. (2018)
Mustard	91	21	127	-	-	-	-	-	-	
Sunflower	108	15	218	-	-	-	5.3	81.4	10.4	Torma et al. (2018); Zhao et al. (2018)
Rape	107	15	218	-	-	-	5.1	65.5	5.4	
Rice straw	0.5- 0.8 ^a	0.07- 0.12 ^a	1.16- 1.66 ^a	41.25	7.03	3.96	-	-	33.6	Torma et al. (2018); Plazonić et al. (2016)

Note: N content, P, K (kg/ ha); OM, C (%); Ca, Mg (cmol (+)/kg); Ash content: (%; dry weight); ^a(%).

Tea and wood residues	N	P	K	Dry matter	C	Ca	Mg	C:N ratio	Ash content	
Tea pruned foliage	252	30	72	7.2	2.9	-	-	11	-	Kamau (2008)
Tea pruned twigs	85	10	21	3.6	1.4	-	-	17	-	
Primary wood	101	28	2	4.2	1.8	-	-	42	-	
Secondary wood	44	13	13	4.2	1.8	-	-	40	-	
Acacia bark	133.4	2.6	8.4	8.9	-	76.5	1.2	-	2.1	Taflick et al. (2015); Van Bich et al. (2018)
Eucalyptus biomass	307.5	28.8	249.3	-	-	-	455.7	131.7	15.4	Reina et al. (2016); Resquin et al. (2020)

Note: N, P, K, Ca, Mg (kg/ ha, dry weight); C (t/ ha).

3. Biochar	N	P	K	Ca	Mg	Total C	pH	C:N ratio	Ash content	
Rice straw biochar at 400 °C	19.8	2.0	24	8.8	5.7	56	8.7	-	39	Naeem et al. (2017)
Wheat straw biochar at 400 °C	19.4	3.8	33	10.3	9.6	62	7.8	-	36	

Pine woodchip biochar at 500 °C	0.7	<0.001	2.1	10.1	2.7	244.5 ^c	8.7	366	-	Brantley et al. (2015)
Rice biochar at 500 °C	0.92 ^a	3.23 ^a	2.48 ^a	875.2	578.9	46.4	11.0	-	34.6	Yan et al. (2021a)
Bamboo biochar at 750-800 °C	0.58 ^a	1.85 ^a	1.01 ^a	560.3	320.6	77.3	11.3	-	5.8	
Peanut biochar at 300 °C	2.6 ^a	-	22.0 ^b	47.4 ^b	45.6 ^b	55.1	9.2	21.5	228.4 ^b	Wang et al. (2014a)
Vermicompost	8.7	<0.1	1.3	26.3	-	181 ^c	8.09	20.9	8.09	Adhikary (2012)

Note: Total N, P, K Ca, Mg, (g/kg); Total C (%); Ash content (%); ^a (%), ^b (cmol (+)/kg), ^c (g/kg).

4. Compost	N	P	K	Ca	OC	pH	C:N ratio	OM	Moisture	
Chicken manure compost	13.19	12.5	20.00	-	325.3	7.92	26.06	72.56	29.9	<i>Li et al. (2021)</i>
Pig manure compost	29.82	15.13	8.16	-	-	8.37	-	73.01	78.89	Li et al. (2012)
Buffalo manure compost	1.3	-	-	-	-	7.3	14	-	-	<i>Doan et al. (2014); Ngo et al. (2011)</i>
Cow manure compost	21.3	10,4	21.7	23.7	-	9.6	-	56.96	29.1	Gil et al. (2008)

Note: N, P, K, Ca (g/kg); OC, OM and moisture (%).

2.5.2 Organic fertilizer and organic tea management practices

Applying animal manure to tea plantation soils could be an effective solution not only for ameliorating soil acidification, improving soil health of tea plantations but also as a waste management tool. Manures from various animals such as sheep, pig, cow and chicken used as organic fertilizers or compost for tea gardens significantly increased pH of acid soils, compared to their chemical nutrient counterparts (Cai et al. 2015; Gu et al. 2019; Ji et al. 2018; Lin et al. 2019; Qiu et al. 2014). For example, Gu et al. (2019) indicated that long-term applications of animal manure resulted in a significant increase of soil pH (5.36), compared to that in non- fertilizer (4.71) and chemical fertilizer practices (4.31). Likewise, application of pig manure over 18 years increased soil pH by 1.1 units (Cai et al. 2015). Additionally, the replacement of chemical fertilizer by organic fertilizer in organic and agroecological tea cultivation has also had positive impacts on soil pH and other soil health indicators (Li et al. 2014; Viet San et al. 2021; Yan et al. 2020). Analyzing more than 2000 tea soil samples collected from conventional and organic tea plantations, Yan et al. (2020) concluded that conventional tea cultivation which employed heavy application of synthetic fertilizers caused severe soil acidification, while organic tea management approach did not result in significant soil acidification. Similarly, our recent study showed that agroecological tea management practices with chicken and buffalo manures as main nutrient supplies significantly improved soil pH compared to conventional tea cultivation which employs intensive chemical NPK (Viet San et al. 2023). As outlined above, the mitigation of acidification of tea plantation soils by organic substance addition could be by alkaline matter and basic cations from added organic fertilizers, which can neutralize the soil acidity (Ji et al. 2018). Moreover, other chemical processes involving manure supplementation such as organic anion decarboxylation and organic N ammonification may play a part in reducing soil acidity (Xiao et al. 2013; Xu et al. 2006). Organic fertilizer can also support soil buffering action, thus reducing soil acidification (Chen et al. 2009). More examples of positive

effects of organic manure and compost usage on soil acidification are indicated in Fig. 7 and Table 4.

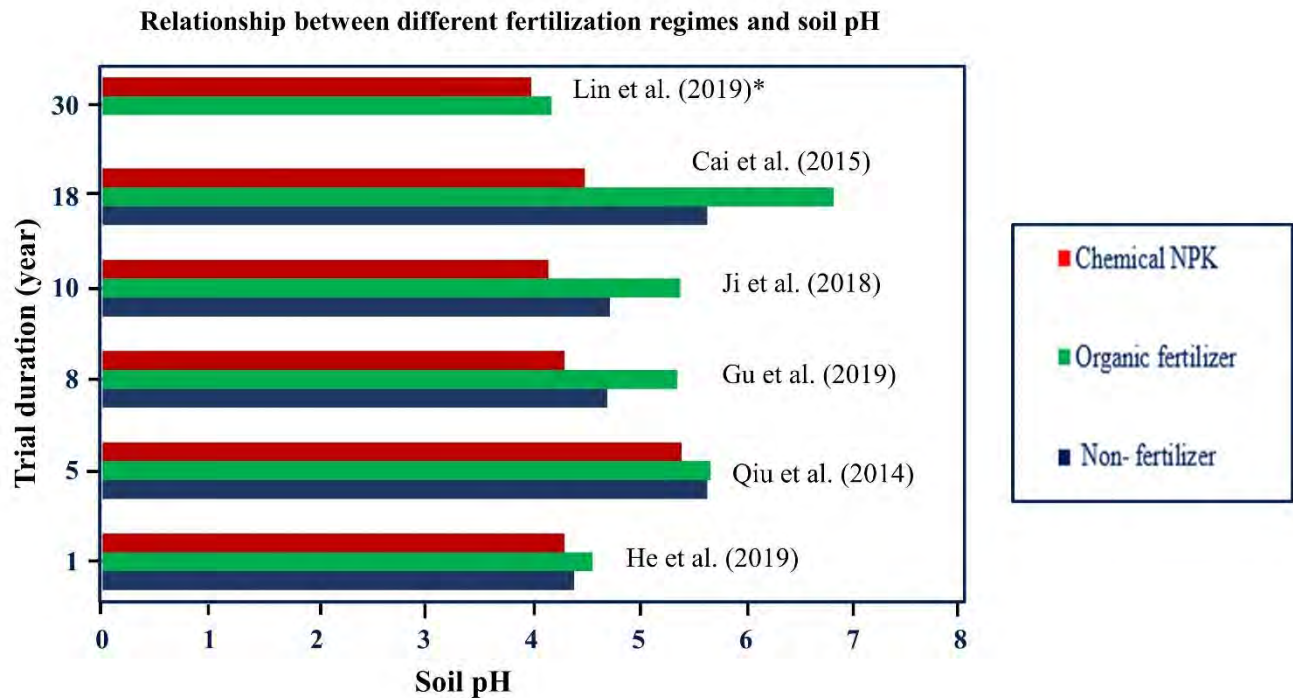


Figure 7. Effects of different fertilizer type applications on soil pH under tea cultivation. Organic fertilization consistently increased soil pH in comparison with chemical fertilizer and non-fertilizer practices. Heavy uses of synthetic fertilizers also led to the highest reduction of soil pH, compared to other fertilization approaches. Adapted from Lin et al. (2019); Cai et al. (2015); Ji et al. (2018) Gu et al. (2019); Qiu et al. (2014); He et al. (2019). (*) the data for non-fertilizer management practice is not available

Apart from ameliorating soil acidification, recycling organic amendments as partial or full substitutes for chemical fertilizers can bring about a range of benefits for other aspects of tea plantation soil health and the environment. Organic fertilizer applications consistently improved soil OM, soil OC, soil exchangeable cations such as Ca^{2+} , Mg^{2+} , Na^+ and K^+ , and nutrient availability,

while reducing risks of Al toxicity, heavy metal accumulation, greenhouse gas emissions and nutrient runoff such as N and P (Table 4) (Cai et al. 2015; He et al. 2019; Ji et al. 2018; Lin et al. 2019; Qiu et al. 2014). Sustainable effects of adopting organic soil amendments in tea plantation soils on biological soil health has been also clearly indicated. Organic materials such as sheep, cow, chicken manures or compost significantly improved soil fauna communities, soil microbial diversity and functional structures (Gui et al. 2021; Li et al. 2014; Lin et al. 2019; Zhang et al. 2020a). Organic fertilizers are naturally rich in nutrients containing more organic matter compared to chemical compounds, thus the replacement of organic amendments provides more organic matter in the soils (Wu et al. 2020; Xie et al. 2019). Richer soil organic contents will attract soil fauna and facilitate the activities of soil microbial communities in converting soil nutrients, which ultimately increase soil nutrient of tea plantation soils (Fan et al. 2017; Xie et al. 2019; Xie et al. 2021). These positive changes in turn will result in increasing soil organism diversity and community structure (Gu et al. 2019; Wu et al. 2020).

There do exist some concerns for recycling animal manure and organic compost which need further consideration. Firstly, organic fertilizer such as rapeseed cake had inconsistent effect on soil pH (Xie et al. 2019; Xie et al. 2021). This discrepancy may result from the dissimilarity of chemical composition of the product and other conditions such as soil type, application rate and management practices (Gu et al. 2019; Wu et al. 2020). Secondly, it has been reported that organic manure cannot ameliorate deep-soil acidification in tea plantations (Li et al. 2016). In this case, biochar or a combined utilization of manure and biochar may be an effective solution to not only mitigate soil acidification but also enhance soil health and tea productivity (Dai et al. 2017; He et al. 2019). Thirdly, long- term application of animal manure and compost to manage acidic tea soils and restore soil health could lead to the risks of heavy metal accumulation and manure- borne pathogen contamination (Cai et al. 2021; Li et al. 2020). For heavy metal contamination, Ji et al. (2018)

indicated that 10 - year application of pig manure did not result in increase of most heavy metals, and Lin et al. (2019) found that sheep manure and rape cake application reduced levels of Cd, Pb and As in soils as well as in tea leaves. To date however, the relationship between animal manure, compost and pathogenic diseases of tea plants has been poorly understood. Thus, an integrated approach including appropriate application rates, reducing chemical inputs and concentrations of heavy metals in animal feed could be all necessary to minimize the environmental risks from using these organic materials as soil amendments and increase their efficacy (Cai et al. 2021; Ji et al. 2018).

2.5.3 Biochar amendment

Among the ameliorants of soil acidification, biochar could be one of the most effective options as it can also improve soil quality, plant productivity, and contribute to a reduction in greenhouse gas emissions (Akhil et al. 2021; Siedt et al. 2020; Zhang et al. 2018). In tea farming, biochar produced from plant residue such as rice, wheat straw and bamboo residues have been commonly incorporated as soil amendment (Chen et al. 2021; Ji et al. 2020b; Wang et al. 2018). Depending on biochar types and application rates, soil condition, tea management practices and the application duration, the liming effect of biochar varied significantly, (Wang et al. 2014a; Yan et al. 2021). As demonstrating in Fig. 8, applying biochar at rates of from 1% to 5% of soil dry weight can significantly increase soil pH from 0.2 to more than 1 units within a few months (Ji et al. 2020a; Oo et al. 2018; Wang et al. 2018; Zheng et al. 2019). Studies conducted in tea plantations also demonstrated the positive outcomes of biochar utilization for correcting soil acidification caused by tea cultivation (Table 4) (He et al. 2019; Ji et al. 2020b; Yang et al. 2021).

Soil pH change

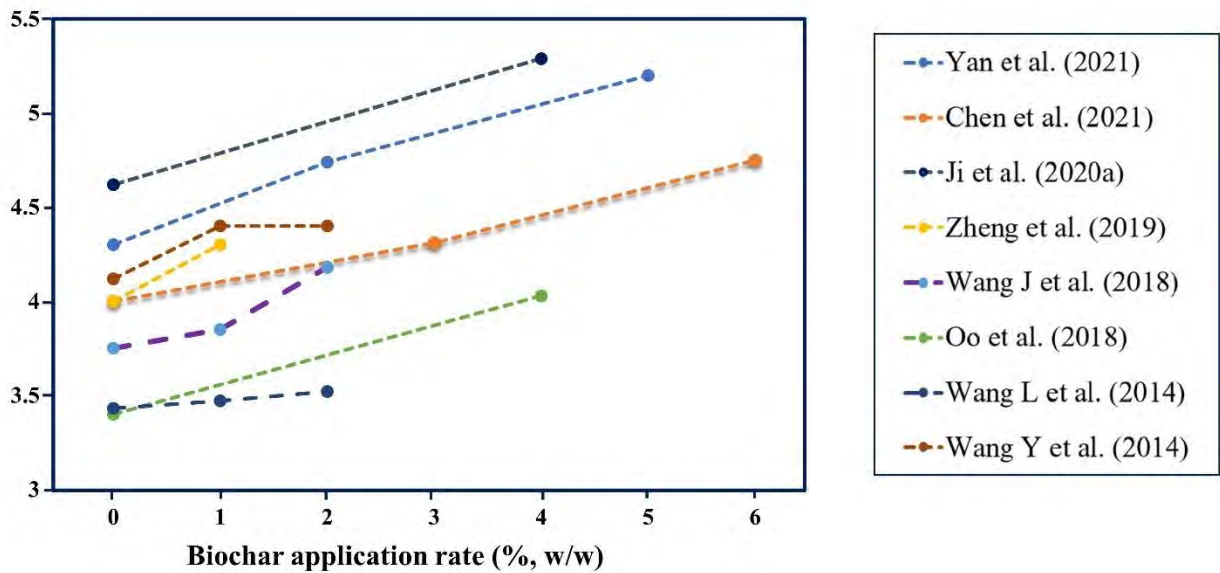


Figure 8. Effects of biochar application rate on pH of tea plantation soils. Data collated from recent publications: Chen et al. (2021); Ji et al. (2020a); Oo et al. (2018); Wang et al. (2018); Wang et al. (2014); Wang et al. (2014) and Zheng et al. (2019)

Biochar ameliorates soil acidification by its natural alkalinity, high pH value and pH buffering capacity. Biochar generally has an alkaline pH value, thus soil amended with this product can become less acidic (Table 3). For instance, a meta- analysis by Dai et al. (2017) indicated that biochar applications significantly increased soil pH by up to 2 units, and in most cases, the pH of biochar is greater than 7.0, which is at least 1.5 units higher than the pH in acid soils. Moreover, mineral constituents of biochar including basic cations such as Ca, Mg, K, Na and alkaline oxides that originated from feedstocks can mitigate soil exchangeable acidity (mainly H^+ and Al^{3+}) in the soil and ultimately increase soil pH (Dai et al. 2017; Patra et al. 2021; Yuan et al. 2011). In addition, soil pH buffering capacity is an important factor contributing to biochar amelioration of soil. Shi et al. (2019) illustrated that rice straw and peanut straw biochar application increased pH buffering capacity by 22% and 32% respectively. It has been verified that the increases in CEC of the soil by

biochar incorporation, driven by protonation- deprotonation processes, was the main mechanism of increasing soil pH buffering capacity (Shi et al. 2017; Xu et al. 2012). Biochar application also suppressed soil nitrification by limiting the availability of NH_3 or NH_4^+ for oxidation because of the surface adsorption or increased emissions of NH_3 due to enhanced soil pH (Wang et al. 2018; Yang et al. 2015). This in turn generally reduces the proton (H^+) released into soil and ultimately increases soil pH (Shi et al. 2019).

Biochar addition also enhanced soil quality indicators, tea growth and productivity, as well as reduced the environmental risks from pollution by heavy metals and greenhouse gases such as CO_2 , N_2O and NO (Chen et al. 2021; Ji et al. 2020a; Yan et al. 2021). Consistently, biochar incorporation in soil improved soil OC, soil nutrient availability including Ca, Na, Mg, P and K contents, soil total N and C (Yan et al. 2018; Wang et al. 2014; Zheng et al. 2019). While the impact of biochar on soil fauna has been poorly investigated, this carbon-rich material has significant effects on enhancing soil microbial diversity and community structure (Table 4) (Ji et al. 2020a; Yang et al. 2021; Zheng et al. 2019). Biochar itself is a source of nutrients, including microminerals, trace elements, ash and so on, so its application also supplies essential agronomic benefits to farmers (Rawat et al. 2019). More importantly, biochar can absorb fertilizers and slowly release these into the soil, which helps to not only retain the nutrient availability in the soil but also reduce fertilizer leaching and drainage, which then contribute to environmental pollution (Rawat et al. 2019). Since soil pH and nutrient status has a close correlation with soil microorganism, the changes in soil chemical and physical properties as a result of biochar application could be the key driven factor for the alteration of soil biological properties (Cheng et al. 2019; Yang et al. 2021).

Several downsides of biochar incorporation need to be considered to improve its effectiveness and reduce the detrimental effects on the environment. Biochar has been considered as the most

expensive soil management solution, particularly for large-scale use in agriculture (Siedt et al. 2020). Since the application rate of biochar normally ranges from 10 to 150 tons/ha and controlling strongly acid soils may require large quantity of biochar, which leads to an increased costs for energy inputs, feedstocks, transportation and incorporation (Dai et al. 2017). Furthermore, most studies on biochar application for managing soil acidification in tea farming to date have been conducted in controlled conditions in China, suggesting that further research either in long-term field conditions or in other tea producing areas would be needed. Overall, biochar indicates a great potential in ameliorating soil acidification and improving tea plantation soil health, however, more comprehensive, and reliable evidence should be provided to validate these advantages.

2.5.4 Plant residues as organic mulching practices

Organic mulching practices employing plant residues and other agricultural wastes have received limited attention to date. Some studies conducted on tea fields indicated that mulching materials such as Fern (*Gleichenia linearis*) and tea pruning materials can alleviate soil acidity (Cu and Thu 2014a; b). Other materials such as crop straws and legume residues also had positive effects on increasing pH of tea plantation soils, either in field or laboratory trial conditions (Table 4) (Wang et al. 2009; Xianchen et al. 2020). In contrast, there have been a number of investigations revealing the negative impacts of organic mulching on soil pH from other cropping systems. Otero-Jiménez et al. (2021) found that rice straw mulch and rice straw burning significantly reduced soil pH by 0.55 and 0.19 units respectively, and the application of wheat straw mulching reduced soil pH by 0.11 units (Mehmood et al. 2014). Finally, some studies have demonstrated that plant residues have no significant effects on soil pH (Iqbal et al. 2020; Ni et al. 2016). Positive effects of crop residues in increasing soil pH could be mainly due to the decarboxylation of organic anions, which can neutralize soil exchangeable H^+ and Al^{3+} , and also reduce the toxicity of Al species to plant roots (Dai et al. 2017). Declines in soil pH following application plant residue mulches could be attributed

to the release of H^+ from nitrification of NH_4^+ , which is produced during the mineralization of organic N in the residues (Dai et al. 2017). Decomposition of crop residues may also produce some organic and carbonic acids, potentially causing soil acidity (Arafat et al. 2020).

The potential of crop residue mulching in enhancing other soil health indicators has been widely recognized. Plant residues improve soil moisture content, soil structure and regulate soil temperature, support soil microbial activities and improve soil nutrient availability, as well as suppress weeds and reduce soil erosion, all of which contribute to enhance soil health and crop productivity (Chatterjee et al. 2017; Kader et al. 2017; Ngosong et al. 2019). These benefits have also been demonstrated in tea cultivation systems. Covering the surface of tea plantation soils with rice straw and tea pruning residues significantly reduced soil temperature variation, soil compactness and soil bulk density, while increasing soil water retention and soil moisture (Cu and Thu 2014a; Xianchen et al. 2020). Organic mulches can also enhance soil nutrient availability (Ca^{2+} and Mg^{2+} , available N, P, K) soil OM content but reduce soil Al^+ concentration (Cu and Thu 2014a; Wang et al. 2009; Xianchen et al. 2020). Enrichment of soil microbial diversity and community structure as a result of mulching material addition have been reported in these studies (Cu and Thu 2014a; b) (Table 4). Organic mulch cover creates favorable moisture and thermal regimes in soils by controlling surface evaporation rates and altering soil temperatures, by reducing temperature in the summer and raising it in the winter (Kader et al. 2017). Under appropriate soil microclimatic conditions, plant litter can decompose and add nutrients to soil. Plant residues and other organic mulch materials generally contain higher level of nutrients compared with inorganic mulch materials, but the influence of organic mulching application on soil nutrients has been also determined by other factors such as soil characteristics, climatic conditions (Iqbal et al. 2020; Kader et al. 2017). In addition, soil physicochemical conditions including soil moisture, soil temperature and soil nutrients play a crucial part in governing soil organisms (Kader et al. 2017; Onwuka and

Mang 2018; Tan et al. 2018). For example, Brockett et al. (2012) concluded that soil moisture is the major factor affecting the community structure of soil microbes as well as enzyme activities. Examples of plant residue mulching and the summary of beneficial impacts of organic mulching, organic fertilizer and biochar applications in tea plantation soils are shown in Fig. 9.

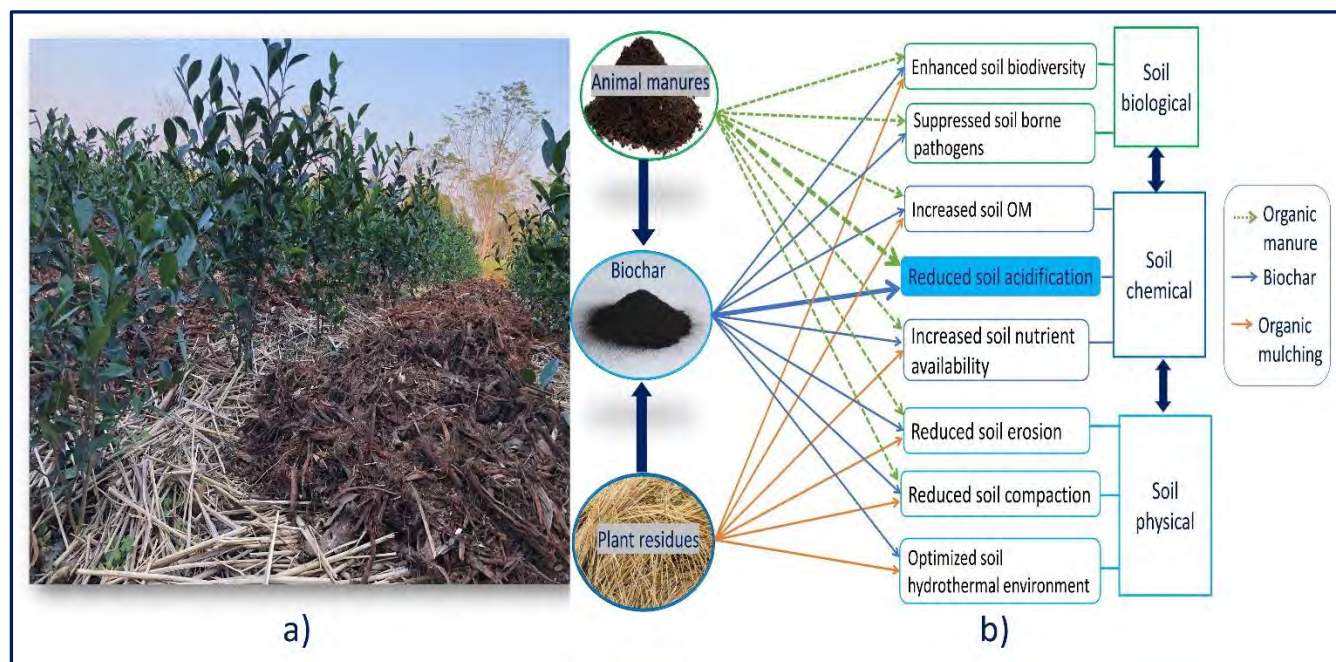


Figure 9. Plant residues (rice straw, Acacia bark and woodchips) and organic manure (poultry manures) applications in tea plantations (a) and summaries of the beneficial effects of some soil amendments derived from agricultural wastes on soil properties of tea plantations (b). Photo was taken in Thai Nguyen province, Northern Vietnam by the author

However, some mulching materials such as crop straws generally decompose quickly, thus need to be frequently incorporated for long-term use. This may require extra labour and investments, preventing farmers from adopting them in the long run (Amoah-Antwi et al. 2020; Dai et al. 2017). Extensive use of plant residues such as tea pruned litter to mulch tea soils could also lead to a decrease of soil pH and the accumulation of active allelochemicals, which can cause soil sickness and tea growth deterioration (Arafat et al. 2020). Too much organic mulch could also result in other

issues such as excess moisture and nitrogen, pests and anaerobic conditions, damaging the plant root and negatively affecting its growth and productivity (Iqbal et al. 2020; Kader et al. 2017). Overall, organic mulching employing plant residues is an effective soil management tool to improve soil physicochemical properties, but its role in controlling tea soil acidity needs further investigations.

2.5.5 Intercropping and agroforestry

Tea plants intercropped with loquat, waxberry and citrus significantly improves soil pH, organic matter, N, P and K availability, tea quality indicators, and reduces soil heavy metal concentrations compared with monoculture tea gardens, regardless of sampling seasons (Wen et al. 2019). Similarly, Xianchen et al. (2020) found that inter-planting of *Vulpia myuros* at the density of 22.5kg/seeds/ha in tea plantations significantly increased soil nutrients (OM, available N, P, K), soil water holding capacity while reducing soil temperature fluctuations and soil compactness at all observed soil depths (0-10 and 10-20cm). In terms of soil organisms, intercropping adoption in tea cultivation enriched soil enzyme activity and regulated tea pests (Xianchen et al. 2020; Zhang et al. 2017) (Table 4). In addition, tea – Ginkgo tree (*Ginkgo biloba* L.) agroforestry significantly increased soil pH (5.86 vs 5.21), soil organic carbon (17.92 vs 16.38 and total N (1.91 vs 1.79) compared with single tea plantations (Tian et al. 2013). The increase of soil pH in the Ginkgo – tea agroforestry is likely due to the alkaline matter formed during the decomposition of Ginkgo tree residues which neutralizes soil acidity (Tian et al. 2013). Intercropping and agroforestry might increase overall ecosystem productivity and nutrient retention by increasing species diversity, increase soil organic matter by plant residues, attribute to the decomposition of fine roots in the deep mineral layers and surface leaves of trees (Brooker et al. 2015; Cong et al. 2015; Dollinger and Jose 2018). Among these impacts, organic matter enrichment could play a key role, containing basic cations and contributing to increasing the supply of important nutrients (Cardinael et al. 2020; Dollinger and Jose 2018).

Table 4. Summary of current studies of organic fertilizers, biochar, plant residues and other relevant options on mitigating soil acidification and improving soil health, tea plant growth, and reducing environmental risks

Material/ Practice	Soil type Location	Experiment type Application rate/time	Soil pH effect	Other positive and/or negative impacts on soil, tea plants and the environment	Reference
Sheep manure + rape cake	Red soil China	- Field experiment - Trial time: 30 years	- Organic fertilizers resulted in an increase by 0.2 units (4.2 vs 4.0) compared to chemical fertilizers.	- Significant increased soil bacterial abundance, total K, while decreasing the contents of Cd, As and Pb in the rhizosphere and tea leaves. - Reduced soil total N (0.23 g/kg); total P (1.24 g/kg).	Lin et al. (2019)
Pig manure	Red soil (Ferralic Cambisol) China	- Field experiment - Trial time: 18 years	- Increased by 1.1 units after 18 years of pig manure application.	- Pig manure application reduced exchangeable Al^{3+} and significantly increased soil exchangeable Ca^{2+} , Mg^{2+} , Na^{+} and K^{+} .	Cai et al. (2015)
Cow manure + Pig manure	Haplic Acrisol Chia	- Field experiment - Manure: 1000-2,000kg/ha - Trial time: 1 year	- Soil pH value with chicken and pig manure practices were 5.36 and 5.09 respectively, compared to 4.71 of non-fertilization and 4.31 of mineral compound (NPK) application.	- Organic fertilizer application increased soil microbial diversity by 8.59–33.14% and resulted in an improvement of potential ecosystem function compared with synthesized fertilizer. - Increased total P but decreased total N.	Gu et al. (2019)
Pig manure	Red soil China	- Field experiment - Substitution of 25%, 50%, 75% and 100% N by organic manure - Trial time: 10 years	- 0.66 unit increased by application of 100% N substitute compared to the non-fertilizer plots - 1.23 units higher compared to the pH value of synthetic fertilizer use.	- Significantly increased soil OC, total N, NH_4^{+} -N contents, available P and K. - Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), soil bacterial diversity and community structure were improved significantly.	Ji et al. (2018)

Cattle manure	Planosols (Clay loam) China	- Field experiment - Manure + biochar, 20,000 kg/ha - Trial time: 2 years	- Organic fertilizer and biochar application resulted in greater soil pH compared to chemical fertilizer.	- Cattle manure and biochar applications reduced NO emission. - Adding cattle manure as a partial substitute for biochar reduced NO emission, and solely biochar application reduced N ₂ O emission by 14%.	Han et al. (2021)
Chicken manure	China	- Field experiment - 11,400kg/ha - Trial time: 5 years	- Chicken manure application resulted in the highest soil pH (5.67), compared to non-fertilization (5.64) and mineral compound (NPK) (5.40).	- Significantly increased soil OM, total N and P; available N, P and K. - Organic manure promoted bacterial diversity, while that was reduced by chemical fertilizer application.	Qiu et al. (2014)
Rapeseed cake	Yellow brown China	- Field experiment - 1.904, 3.928, 6.207 kg/ha - Trial time: 1 year	- Rape seed cake (6,207 kg/ha) decreased soil pH by 0.19 units while with chemical fertilizer was 0.33 units.	- Soil OM, available P and K increased by 31.4%, 26.2%, and 21.7%, respectively - Increased restoration of NH ₄ - N, NO ₃ -N, total P and K contents in soil while reduced the substances in runoff water.	Xie et al. (2019)
Cow manure	Brown loamy China	-Field experiment - 20 tons/ha - Trial time: 6 months	- Data not provided	- Significantly increased the relative abundance of <i>Proteobacteria</i> and <i>Bacteroidetes</i> species and enhanced the diversity of bacterial communities.	Zhang et al. (2020b)
Rapeseed cake	Acid yellow brown China	- Field experiment - 1,708, 4,270, 6,831 and 8,539 kg/ha/year - 8 months	- Significantly increased soil pH by 2.19 – 4.29% compared to chemical compound treatments.	- Increased total OM and preserved soil C and N pools of the tea plantations - Reduced the nitrogen inputs (NH ₄ - N and NO ₃ -N) in the tea plantation runoff.	Xie et al. (2021)
Pig, chicken and cattle manure compost	Alfisol China	- Field trial - Trial time: 1 year	- Soil pH for pig, chicken and cattle manure compost uses were 4.56, 4.48 and 4.57, respectively, compared to 4.44 of non-fertilizer and 4.31 of chemical fertilizer practices.	- Increased soil OC, total N while reducing N ₂ O and NO emissions. - Organic fertilizer has no influence on tea yield, but that was increased by chicken manure and biochar combined application.	He et al. (2019)
Organic	Ferralsol	- Field trial	- Organic tea management with	- Increased soil OM, soil N and C/N ratio.	Li et al.

management (Chinese Pennisetum, rape cake and farmyard manure)	China	- Chinese Pennisetum: 4,000kg/ha; rape cake: 3,000kg/ha; farmyard: 2,000kg/ha/year - Trial time: 6 years	organic fertilizer uses resulted in greater soil pH compared to conventional tea management; but lower compared to natural tea plantations.	- Enhanced species diversity, species richness and trophic diversity of nematodes in the soil.	(2014)
Organic management (rape cake, compost, and commercial organic fertilizers)	Ultisols China	- Field experiment - 4,500- 9,000 kg/ha/year - Trial time: around 10 years	- Soil pH has an inconsistent correlation with tea management methods.	- Increased soil microbial C by 164.4% and soil microbial N by 482.9% on average. - Total OC, N and available P increased significantly in organically managed tea plantation soils, but Ca and Mg availability decreased in comparison with conventional management.	Gui et al. (2021)
Agroecological management (chicken and cow manure as main nutrient supplies)	Ferralic Acrisols Vietnam	- Field experiment - 6,000- 8,000 kg/ha/year - Trial time: 5-10 years	- Increased soil pH by 0.35 units on average, compared to conventional tea plantations.	- Significantly improved soil OM, colonization and intensity of arbuscular mycorrhizal fungi (AMF). - Reduced soil total N.	Unpublished data
Organic management (cow and pig manure, commercial organic fertilizer)	Red soil China	- Field experiment - Management duration: 14 years	- Soil pH increased by 0.91 units compared to conventional tea plantations, and 0.06 units compared with the tea plantations employing a combined application of organic and chemical fertilizers (non-polluted management practices).	- Increased total OC, available P, NH ₄ -N and NO ₃ -N but total P and N were lower than that in the non-polluted tea management). - Improved soil microbial diversity, increased the abundances of beneficial soil microbes, and altered the interaction network structure compared with conventional and pollution-free management practices.	Tan et al. (2019)
Organic management	Bangladesh	- Field research	- Soil pH of organically managed tea plantations was 5.1, compared to 4.2 of conventionally managed tea plantations.	- Increased total OM and nutrient availability (K, Ca, Mg, P, Zn and S) - Significantly increased tea yield and economic efficiency.	Sultana et al. (2014)

Organic management (Sheep manure)	Laterites China	- Field research - 6.000kg/ha/year, dry matter - Management time: 3 years	- Soil pH was significantly lower compared to that in longan orchard, both in the surface (5.05 vs 5.32) and 10-20cm depth (5.04 vs 5.24). - No significant difference compared to conventional tea management plantations.	- Organic tea management increased soil P availability, enhanced soil microbial communities (bacteria, fungi, actinomycetes and AMF) compared to conventional tea management. - Conversion of longan to tea plantation significantly reduced soil fertility.	Wu et al. (2020b)
Rice straw biochar	Oxisols China	- Laboratory incubation - 1%, 2% and 5% of the dry soil weight (w/w) - Trial time: 21 days	- Soil pH was 4.4; 4.2 and 3.9 for 5%, 2% and 1% of biochar applications respectively) - Soil pH significantly increased by biochar application, but that was lower compared to lime (CaO) application.	- Nitrification would be detrimental to the N uptake of tea, while NO ₃ -N produced from nitrification could be lost by leaching, runoff and denitrification. - Tea soil pH should be maintained at higher value than the optimum pH for nitrification (~5.1)	Wang et al. (2018a)
Rice husk biochar at 550 °C	China	- Laboratory incubation - 0.5%, 1%, 2% (w/w) - 60 days	- Application of biochar at 2 and 4% significantly increased soil pH (3.52 and 3.63 respectively).	- The incorporation of fast pyrolysis rice husk led to a significant increase of soil total C, N, extractable Ca, Na, Mg and K contents, while available Al and Pb were reduced.	Wang et al. (2014b)
Rice, wheat and peanut residue biochar at 300 °C	Ultisol China	- Laboratory incubation - 1%, 2% (w/w) - Trial time: 65 days	- Soil pH increased in all biochar application treatments, and the highest soil pH value was observed in peanut biochar, followed by wheat and rice residue biochar.	- Significantly increased soil exchangeable acidity but reducing soil exchangeable Al and - Increasing biochar application rate has no further effect on soil pH. - Reduced acidity produced from N cycle.	Wang et al. (2014a)
Rice straw biochar at 550 °C; Bamboo straw biochar at 750-800°C	Loamy clay China	- Glasshouse trial - 2% and 5% (w/w) - Trial time: 1 year	- pH increased by 0.9 units by bamboo biochar application, 1 unit (from 4.30- 5.30) by rice biochar use at the rate of 5%. - Increasing biochar additional	- Increased plant nutrients (P, K and Mg concentrations), while reducing Mn and Cu concentrations. - Significantly improved tea growth characters compared to conventional tea management	Yan et al. (2021a)

			rate resulted in greater soil pH increase.	without biochar. - Rice and bamboo biochar have no significantly different effect on tea growth and tea soil nutrients.	
Tea pruning residue biochar at 500- 600°C	Red- yellow Japan	- Laboratory incubation - 4% (w/w) - Trial time: 90 days	- Biochar amendment significantly increased soil pH at the surface (0-5 cm, 0.23 units) and 5- 10 cm soil layer (0.73 units).	- Tea pruning residue use as mulch significantly increased soil total N, C, and also N ₂ O and CO ₂ emissions. - Converting tea pruning residue to biochar amendment and its incorporation significantly mitigate N ₂ O emission by up to 74.2%, but increased CO ₂ emission.	Oo et al. (2018)
Bamboo residue biochar at 500 °C	Inceptisols	- Glasshouse trial - 3% and 6% (w/w) - Trial time: 180 days	- Soil pH increased by 0.31 units with an application rate of 3%, 0.75 units with incorporation rate at 6%.	- Reduced NH ₄ ⁺ -N leaching by up to 91.9%; NO ₃ ⁻ - N by a maximum of 66.9% and total N by up to 72.8%. - Enhanced soil nutrient retention (N by up to 23.9%). - Improved soil microbial biomass and enzyme activity.	Chen et al. (2021)
Wheat straw biochar at 450 °C	Plinthosols China	- Laboratory incubation - 4% (w/w) - Trial time: 35 days	- Soil pH increased 1.09 units compared to non-fertilizer practices, but lower compared to the combined application of biochar and N fertilizer (5.2 vs 5.4).	- Biochar amendment increased the abundance of ammonia oxidizing bacteria and Nitrous oxide reductase genes. - Increased soil C/N ratio and decreased N ₂ O emission in acidic soil. - Biochar could increase N ₂ O emission in alkaline soils	Ji et al. (2020a)
Legume and non-legume biomass at 500 °C	Udisols China	- Laboratory incubation - 1% (w/w) - Trial time: 30 days	- Soil pH immediately increased by around 0.4 units after biochar addition, then remained stable. - Legume biochar has a greater impact on increasing soil pH	- Increased soil dissolved OC but reduced inorganic N. - Suppressed N ₂ O emission by around 40% - Significantly altered fungal community structure, relative abundance of Ascomycota community, but has no significant effect on	Zheng et al. (2019)

			compared to that of non-legume biochar.	bacterial community.	
Wheat straw biochar at 450 °C	Plinthosols China	- Field experiment - 20,000kg/ha - Trial time: 2 years	- Significantly increased soil pH by 0.2 units.	- Biochar application decreased N ₂ O and NO emissions from acidic tea soils. - Denitrification was mainly responsible for producing N ₂ O in acidic soil. - Nitrification and denitrification processes were both facilitated by biochar addition.	Ji et al. (2020b)
Wheat straw biochar at 450 °C	Alfisol China	- Field experiment - 7,500 kg/ha - Trial time: 1 year	- Increased soil pH by 0.68 units compared to conventional chemical N, and by 0.55 units compared with non-fertilizer treatment.	- Biochar applications reduced N ₂ O and NO emission factor by 1.82 and 1.38 respectively, compared to chemical N use. - Biochar combined with manure chicken applied to tea soils could mitigate N gas emissions and increase tea productivity.	He et al. (2019)
Mushroom residue biochar at 500 °C	Ultisols China	- Field experiment - 1,350 kg/ha and 2,390kg/ha - Trial time: 1 year	- Biochar application at a rate of 1,350 kg/ha increased soil pH by 0.1 units after one year, while the figure for the higher rate (2,390kg/ha, biochar + based chemical fertilizer) was 0.27 units.	- Biochar application enhanced plant beneficial fungal genera such as <i>Chloridium</i> , <i>Clavulina</i> , <i>Amylocorticium</i> , <i>Rhodosporidiobolus</i> and bacterial genera such as, <i>Mizugakiibacter</i> , <i>Rhodanobacter</i> and <i>Pedobacter</i> . - Increased tea yield and yield components, tea quality indicators such as amino acids and water extract contents.	Yang et al. (2021)
Rice straw	- China	- Field experiment - 7 cm thick - Trial time: 8 months	- Increased soil pH by 0.13 units compared to non-mulching practice.	- Reduced soil temperature variation and having a significant cooling effect in the deep soil layer - Significantly improved soil water retention while reducing soil compactness. - Significantly increased soil OM, available N, P, K and total N.	Zhang et al. (2020d)
Plant residue ash (canola, wheat rice, corn,	Alfisol China	- Laboratory incubation - 20g ash/ 350g soil	- Plant residue ash significantly increased soil pH (by 0.3 units on average).	- Reduced soil Al exchangeable concentrations.	Wang et al. (2009)

soybean peanut...)		- Trial time: 60 days	- Leguminous residues had more significant effects in raising soil pH than the non-legumes.		
Fern (<i>Gleichenia linearis</i>)	Acrisols Vietnam	- Field experiment - 0, 15, 25, 35 and 45 tons/ha (fresh weight) - Trial time: 3 years	- Application rate of 15 and 25 tons/ ha significantly increased soil pH at the 3 years of experiment, while the rates of 35 and 45 tons/ha had inconsistent effect on soil pH.	- Significantly increased soil basic cations (Ca ²⁺ and Mg ²⁺) while reducing soil Al ³⁺ - Improved soil moisture, soil bulk density and humus substances, and enhanced soil microbial activities. - Application rate at 25tons/ha of fern is recommended.	Cu and Thu (2014a)
Tea pruned residues	Acrisols Vietnam	- Field experiment - 30 tons/ha - Trial time: 3 years	- Tea residue mulches significantly increased soil pH (by 0.3 units after 1 year; 1.1 units after 3 years) compared to no- mulching practice.	- Increased soil moisture, soil OM content and reduced soil bulk density. - Significantly increased total number of soil bacterial, fungi and actinomycetes. - The influences of tea pruned residues on soil properties reduced rapidly after 3 application years.	Cu and Thu (2014b)
Peanut hull	Brown soil China	- Field experiment - 10 cm thick	- Soil pH slightly increased (0.04 units) compared to non-mulch treatments.	- Significantly increased soil moisture contents, OM, total N and K, available N but reduced total P, available P and K. - Increased fungal community diversity in 0–20 cm soils and that of bacterial communities in 20–40 cm soils.	Zhang et al. (2020c)
Intercropping with <i>Vulpia myuros</i>	China	- Field experiment - 7 cm thick - Trial time: 8 months	- Increased soil pH by 0.06 units compared to tea monoculture.	- Significantly increased soil OM, soil available N, P, K and total N, and soil enzyme activity. - Optimized topsoil temperature, increased soil water holding capacity while reducing soil compactness.	Zhang et al. (2020d)
Intercropping with aromatic	Acidic Histosols	- Greenhouse trial - Trial time: 2 years	- Data not provided	- Decreased the population of tea green leafhoppers while increasing the natural enemies	Zhang et al. (2017)

plants (e.g., <i>Cassia tora</i> , <i>Medicago sativa</i>)	China			of tea pests such as spiders, lacewings, and parasitoids.	
Intercropping with fruit trees (loquat, waxberry and citrus)	Yellow soil China	- Field experiment - Trial time: 30 years	- Soil pH at three soil depths (0-10, 10-20 and 20-30 cm) significantly increased by intercropping practices, compared to that in mono tea plantations.	-Increased soil OM, available P and K while reducing heavy metal (Cr, Cd, As, Hg, and Pb) - Improved tea quality indicators such as amino acid and catechin.	Wen et al. (2019)
Agroforestry (tea-Ginkgo tree (<i>Ginkgo biloba</i> L)).	China	- Field experiment - Growing distance: 10 x 10 m and 6 x 6 m - Trial time: 11 years	- Increased soil pH at all observed soil depths (by 0.65 units at 0-10 cm layer, 0.15 at 10- 20 cm layer and 0.35 at 20-30 cm layer).	- Significantly increased soil OC, OM and total N contents, soil microbial biomass, and enzyme activity. - Enhanced soil productivity and sustainability.	Tian et al. (2013)

2.6 Field study materials and methods

2.6.1 Study site description and experimental design

This field study was conducted in 4 neighboring communes including Tan Cuong, Phuc Xuan, Phuc Triu and Quyet Thang, which are in the Northwest border areas of Thai Nguyen city, Thai Nguyen province, the largest tea producing province in Northern Vietnam (Fig. 10). This region is characterized by a tropical monsoon climate, with four distinct seasons with an annual mean temperature of approximately 23°C (Dao et al. 2021). The mean annual precipitation ranges from 1500 – 3000mm, and the peak of the rainy season is from May to September (Xuan et al. 2013). Land areas used for tea production are generally slightly sloping (8-15°), and the soil type is classified as Acrisols according to the FAO/WRB classification system (FAO, 1998). Agroecological tea management practices refer to tea plantations that have received organic manure (chicken, cow and/or buffalo compost, 2.5-3 tons/ha/year) and commercial organic fertilizers (3-4 tons/ha/year), organic mulching (crop straw, wood chips, tree barks and Fern), integrated pest and disease management (IPM/IDM, manual control and biopesticides) as the main pest and disease control method for at least 5 consecutive years to the date of sampling (Table S1, Appendix 4). These agroecological tea plantations were granted the VietGAP certification, a voluntary standard accreditation providing the criteria and requirements for safe and sustainable agriculture production issued by the Vietnamese government (Hoang 2020; My et al. 2017). Since 2017, these tea fields have been in transition to organic tea production, which means that no chemical fertilizers and pesticides have been applied since then to comply with the certification requirement. Conventional tea plantations were subjected to traditional management method, with NPK (3-3.5 tons/ha/year) and urea (100-150 kg/harvest/ha) as main nutrient supplies (Table S2, Appendix 4); chemical pesticides as main pest and disease control method. Each experimental tea plot has the area ranged from 1,000- 5,000 m², and the tea variety is LDP1 (the variety that were crossed between Dai Bach

Tra variety originally imported from China and PH1 variety from India), 6 years-old (2019). In addition, all investigated tea plantations were irrigated regularly using underground water.

2.6.2 Tea production economic efficiency

Primary data concerning economic aspects of tea production were conducted using a household survey over 3 years from 2019-2021, which consisted of 35 households who adopted agroecological tea production and 31 conventional tea producing households from the 4 communes listed above. To ensure the credibility of this study, we have closely collaborated with local agricultural agencies and tea cooperatives to select the most representative tea growing households in the 4 communes, where about 70% of the total tea production areas of the city are produced. Criterion for selecting the representative households for interview included the production areas (at least 1,000 m² for one selective plot), identity of tea variety and tea ages being cultivated (LDP1 variety, 6 years old as of 2019), household investment capacity and labor availability (number of working adults), tea farming experience as well as having equal access to extension services and technological support. The production economic efficiency of the two tea production management systems was compared using the equation as follow:

$$NI = \sum_{i=1}^n R_i - \sum_{i=1}^n E_i$$

Where:

NI is the net income that a tea growing household earns from one hectare (ha) of tea production, either adopting agroecological or conventional management practices.

R_i is the total incomes per ha by selling tea fresh leaves, and any subsidies from government and other agencies for each type of cultivation method (r₁, r₂, ...r_n).

E_i is the total expenses for tea production per ha and any related costs, such as fertilizers, pesticides, labor costs, irrigation equipment, machinery, and other tools (e₁, e₂... e_n).

All the amounts were converted from Vietnam Dong (VND) to USD, adopting the current exchange rate (1 USD = 23,200 VND).

2.6.3 Soil and root sampling and analyses

A total of 20 tea plantations from the 66 households mentioned above (10 agroecological and 10 conventional plantations), were selected in 2019 from the 4 communes (Tan Cuong: 7 agroecological plots; Quyet Thang: 1 agroecological, 5 conventional plots; Phuc Xuan: 4 conventional plots and Phuc Triu: 2 agroecological, 1 conventional plots), with the objective to study the impact of different tea management methods on soil physicochemical and biological properties, as well as tea yield and yield components. Apart from meeting the criterion set out for all 66 plantations, these 20 plots have a minimum area of 1,500 m² and are located within a small area (2.5 km² radius) to reduce the soil variability. Of the 20 tea plots, 10 plots were converted from annual croplands and 10 were original tea soils. Converted lands were used as paddy for multiple years, with one rice growing season per year and other annual crops such as maize, peanut and vegetable. These plots are flat (slope < 10⁰), used for flood irrigation and have been converted to plant tea (1st tea generation) by adding hill soils on top (0.5-1 m deep), which were taken from nearby forest land and share the similar characteristics with non-converted tea soils. Original tea plantation soils were hill soils that have been used for tea plantations for at least 2 tea generations (15-30 years). They are slightly sloping (10-15⁰) with thick topsoil, never been flooded (Table S1-S2, Appendix 4).

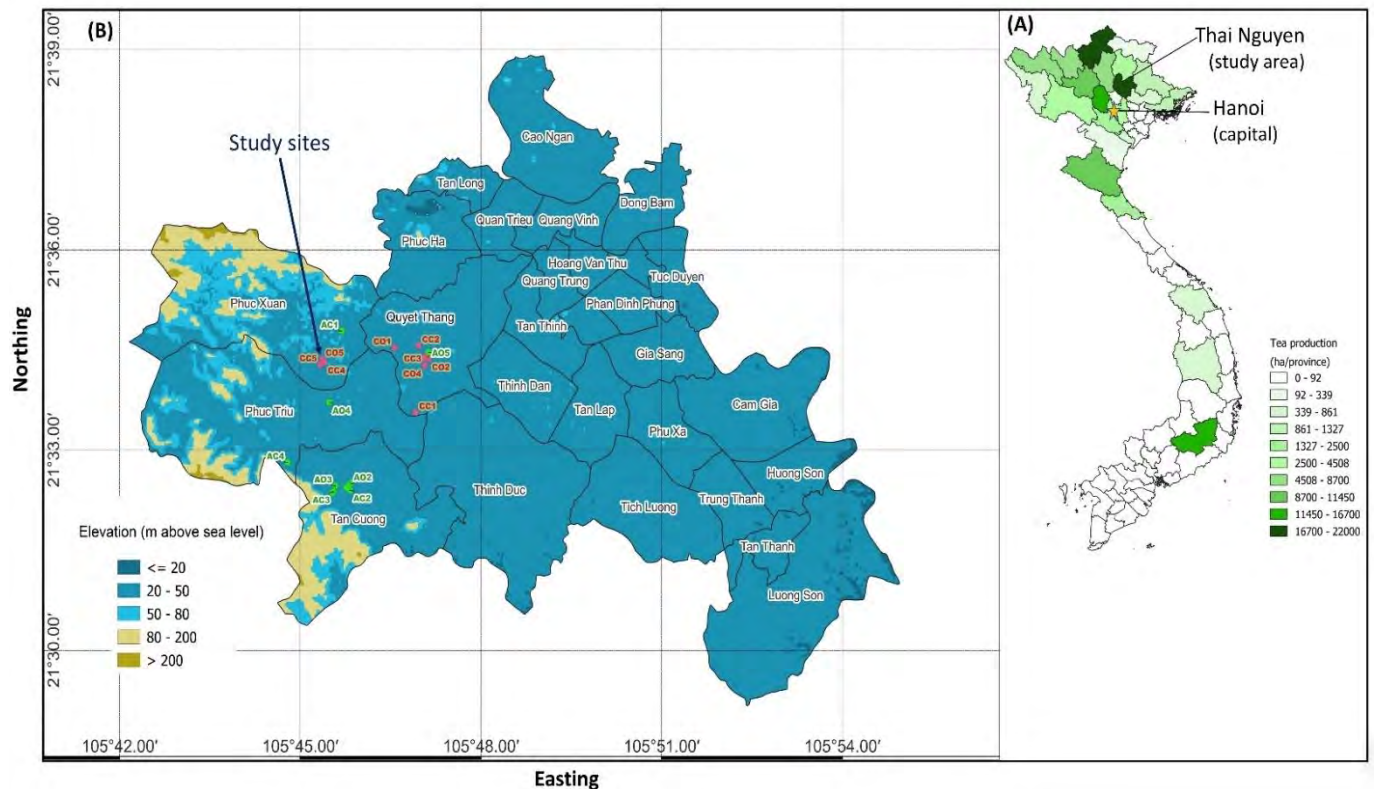


Figure 10. Location of Thai Nguyen province in the Vietnam map with tea production areas as of 2019 (A), and the research sites in Thai Nguyen city, Thai Nguyen province (B). AO1, AO2, AO3, AO4, AO5: Agroecological original plantations; AC1, AC2, AC3, AC4, AC5: Agroecological converted plantations; CO1, CO2, CO3, CO4, CO5: Conventional original plantations; and CC1, CC2, CC3, CC4, CC5: Conventional converted plantations

A sampling area (6m x 9m) was located in the center of each experimental field for conducting soil and root sampling. First, soil macrofauna was collected in the morning to avoid the effect of heat from the sun and other intensive activities such as tea harvesting and other sampling, as some macrofauna retreat quickly. In the center of each sampling area, a soil sample of 20 x 20 x 20 cm was dug, 20-30 cm away from the tea trunk, then all the soil was quickly collected into basins. Soil macrofauna was harvested by carefully hand-sorting the soils and then preserved in 50 ml plastic tubes containing 70% alcohol and then stored at 4°C until being identified to group levels. Likewise, about 200g fresh soil was sampled from holes with dimensions of 10 x 10 x 20 cm and stored in

medium size resealable plastic bag, then immediately stored in cool box containing ice blocks at the fields for analyzing soil mesofauna. These soil samples were then transported immediately after sampling into the lab and stored at 4⁰C. Soil macrofauna and mesofauna were sampled at different dates within the same week.

Following the soil fauna sampling, 12 soil samples were collected per plot, distanced by 3 m in width and 4 m in length from each other. Surface soil samples (0-20 cm deep) were collected, then mixed well and large stones removed. About 500 g of soil was then stored in a large size resealable plastic bag, air dried and kept at room temperature for physicochemical analyses. At the same time, 12 finest tea root samples (30-40 g per sample) were collected within a circle of 1 m from the same points for soil sampling then contained in paper envelopes and air dried for AMF analyses. Soil physicochemical analyses included soil texture (Kilmer and Alexander 1949), soil pH (H₂O) (1:5 Soil: water suspensions), soil OM (Walkley and Black 1934), available Phosphorus (P) (Olsen & Sommers, 1982) and total Nitrogen (N) (Kjeldahl method, as described by Archibald et al., 1958). Fine roots were dried in an oven at 40⁰C following sampling, and AMF staining was implemented using the ink and vinegar method (Vierheilig et al. 1998). The frequency (F%) and the intensity (M%) of AMF colonization were assessed following the technique described by Trouvelot et al. (1986). Generally, fifteen root fragments of 1 cm taken from each sample were observed and the presence and the intensity of colonization were recorded based on the scores (from 0-5) of each fragment.

Soil mesofauna was extracted using two protocols: the heated funnel as described by Edwards (1991) and the modified centrifugal method (Dritsoulas and Duncan 2020). For the funnel method, a thin layer of fine fresh soils (50 g per sample) was spread on a fine sieve or a small plastic basket and applied heat on top for 72 hours. Under the effect of heat, soil mesofauna moved downward and

was collected in the plastic tubes which were tightly connected to funnels and filled with 70% alcohol. For the second method, fresh soil samples (50 g) were initially sieved using mosquito net (mesh size \approx 1 mm) to remove large materials and the fine materials that passed through were then filtered through a 400-mesh sieve to get a bulk subsample containing soil mesofauna and organic matter. The subsample was continuously filtered through a 38-mesh sieve, discarded any materials that passed through and collected the remaining materials into 2-4 centrifuge tubes (total volume \approx 50 ml), and centrifuged at 1700 revolutions per minute (RPM) for 5 minutes to remove organic debris and precipitate soil mesofauna and soil particles in the decanted supernatant. The subsample was then filtered again with the 38-mesh sieve, and the remaining materials were mixed with sucrose solution (1.3M) and centrifuged (1700 rpm, 1 min) to suspend soil mesofauna in the supernatant for collection. Soil mesofauna were then preserved in 70% alcohol solution and identified at the group level, using a dissection microscope. Soil macrofauna extraction was undertaken within a week from the time of soil sampling.

2.6.4 Tea yield, yield component and quality measurement

Tea yield and yield components in the two production systems were compared for 3 consecutive years, from 2019- 2021. In the region, tea growers usually conduct 8 harvests per annum, starting in late February/early March and ending in late November/December with an interval of 30-45 days between harvest, depending on the seasons. The present research was conducted in LDP1 variety, which will be 9 years old in 2022 and is in the middle of its life cycle. Tea yield components including density of tea shoots/m² and average weight of a shoot were measured by randomly placing a quadrat (1 m x 1 m) at the center of each trial plot during the harvest days then manually picked all tea shoots presenting in the quadrats, with 5 replicates per plantation. All harvested tea shoots (1 bud and 2 leaves) were then counted to assess the density, and 100 tea shoots were

randomly selected for assessing their weight. Tea yield (tons of fresh leaves/ha/year) was measured by recording the weight of all fresh tea shoots harvested from the research sites from 2019-2021.

In 2021, a total of 60 samples were randomly picked by tea farmers from the 20 selected tea plantations, each containing approximately 500 g of fresh tea leaves (one bud and two leaves). After being harvested, the green tea samples were immediately sent to the Northern Mountainous Agriculture and Forestry Science Institute (NOMAFSI) for processing and sensory assessment, adopting the standard TCVN 3218-2012 issued by the Ministry of Science and Technology of Vietnam in 2012 (Cuong 2011; Luyen et al. 2014). Fresh tea samples were processed as follows: Light wilting → Enzyme destruction by drying in a barrel rolling → Rub → Drying in barrel-rolling → Final green tea product, all of which were undertaken at the Tea Research and Development Center (NOMAFSI). Afterwards, a recognized panel of 9 highly trained and experienced members (4 females and 5 males), who are mainly senior tea researchers from NOMAFSI, were recruited to take part in the sensory evaluation, which was conducted in a panel room (22°C ±1, free of food/drink odors, fluorescent lighting) for evaluating and presenting marks for the intensity of the target tea quality attributes, including appearance of dried tea leaves, color, smell, and taste of the tea brew. In the test, 3 g of each dried tea sample were coded with 3 digits in random order and served to each panelist simultaneously for evaluating the appearance of the dried tea. In the meantime, a tea infusion was prepared by putting 3 g of the same dried tea into 150 ml boiled water (93-95°C) in 5 minutes, and then the mixtures with the same codes as the dried samples were served and the sensory properties were evaluated. The panelist could then discuss the selected representative descriptors for each attribute according to the standard TCVN 3218-2012, then independently decide the marks for each attribute, using the five scale marks in which 5 is the highest mark given to the best attribute and 1 mark is for the poorest attribute. The average marks of

each sensory attribute were based on the marks given by 9 panelists, and the overall marks were calculated using the equations:

$$D = \sum_{i=1}^4 D_i \cdot k_i$$

Where:

D is the overall marks used to calculate the final grade of the tea quality as follow: Very good: 18.2-20; Good: 15.2-18.1; Moderate: 11.2- 15.1; Poor: 7.2- 11.1 and Failed: ≤ 7.1 .

D_i is the panel average marks of the attribute i (appearance, color, taste, and smell).

k_i is the important index for the attribute i as follow: appearance (1 or 25% if by percentage), color (0.6 or 15%), taste and smell (both 1.2, or 30%).

2.6.5 Statistical analyses

Data used in this study was analyzed using Microsoft® Excel, XLSTAT (Addinsoft 2016) and R software. Comparison data of economic efficiency between conventional and agroecological cultivation methods was subjected to one-way Analyses of Variance (ANOVA), while the different effects of cultivation management and land conversion practices on tea root AMF colonization, tea soil fauna compositional communities, tea yield and yield components, as well as sensory indicators were determined using two-way ANOVA. Soil physicochemical data were $\ln(x)$ transformed and the normal distribution verification was performed before two-way ANOVA. To examine the differences between levels within each factor, Tukey-HSD tests were performed for post-hoc comparisons. In addition, a principal component analysis (PCA) was employed to assess the correlations between the soil characteristics and the mycorrhization indicators. Furthermore, soil fauna diversity indexes were performed using the *vegan* package in R version 4.0.3 (Oksanen et al. 2013).

2.7 Results

2.7.1 *Production economic efficiency*

Table 5 compares the economic indicators between the agroecological and conventional tea production systems from 2019-2021 in 4 communes in Thai Nguyen city, Northern Vietnam. Overall, agroecological tea production requires significantly more inputs but provides significantly higher incomes for the adopters. Agroecological management requires investments in organic fertilizers (USD 5,215), pesticides (USD 679) (Table S1, Appendix 4) and labor cost (USD 6,401) per hectare of tea. In comparison, the expenses of conventional tea farmers in the same categories were significantly lower (USD 3,368 for fertilizers, USD 482 for pesticides and USD 4,581 for labor cost). Similar trend was also observed in other costs (irrigation equipment, machinery, tools for growing and harvesting etc.), where agroecological tea households needed to spend more than USD 770 year⁻¹ ha⁻¹, compared with USD 605 invested by conventional tea growers. In total, farmers producing organic tea need to invest US 13,000 ha⁻¹, those producing conventional tea invest around USD 9,000 ha⁻¹. However, households who adopted agroecological tea cultivation method made significantly more money at the end of the year, which accounted for around USD 24,000 (year⁻¹ ha⁻¹) compared to the non-adopters (USD 15,636 year⁻¹ ha⁻¹). This was mainly attributed to the difference in selling prices of fresh tea leaves, as the average price for conventional tea products was around USD 1 lower than that for the agroecological tea products for each kg (USD 1.7 vs 2.78). In addition, agroecological tea growers have been subsidized by either local government agencies or organic fertilization companies, worth around USD 411 (year⁻¹ ha⁻¹), mainly via supplies of commercial organic fertilizers without any cost or with low interests. The aim of this initiative is to promote sustainable tea and other crop production in the province and country, which was not available for conventional tea production.

Table 5. Comparison of economic efficiency of the agroecological and conventional tea production systems from 2019-2021 in Northern Vietnam

Indicators/Year	2019		2020		2021		Mean (2019-2021)	
	Agroecological	Conventional	Agroecological	Conventional	Agroecological	Conventional	Agroecological	Conventional
Area (ha ⁻¹)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Fresh yield (tons year ⁻¹ ha ⁻¹)	14.65	15.84	14.22	15.44	14.29	15.25	14.38 ^b (1.12)	15.51 ^a (0.91)
Price (USD kg ⁻¹)	2.65	1.65	2.55	1.60	2.45	1.52	2.55 ^a (0.26)	1.59 ^b (0.15)
Subsidy (year ⁻¹ ha ⁻¹)	425.00	0.00	425.00	0.00	385.00	0.00	411.67 ^a (23.09)	0.00 ^b (0.00)
Revenue (USD)	39,247.00	26,136.00	36,686.00	24,704.00	35,395.50	23,180.00	37,109.67^a (2,654.90)	24,673.33^b (2,485.42)
Fertilizers (year ⁻¹ ha ⁻¹)	5,323.00	3,889.00	5,158.00	3,275.00	5,165.00	2,941.00	5,215.33 ^a (480.6)	3,368.33 ^b (427.84)
Pesticides (year ⁻¹ ha ⁻¹)	685.00	485.00	682.50	496.00	670.50	465.00	679.33 ^a (56.09)	482.00 ^b (25.71)
Labor cost (year ⁻¹ ha ⁻¹)	6,638.00	4,680.00	6,240.00	4,575.00	6,325.00	4,490.00	6,401.00 ^a (209.60)	4,581.67 ^b (95.17)
Other costs (year ⁻¹ ha ⁻¹)	836.00	620.00	755.00	606.00	722.00	589.00	771.00 ^a (58.66)	605.00 ^b (32.89)
Total (USD)	13,482.00	9,674.00	12,835.50	8,952.00	12,882.50	8,485.00	13,066.67^a (880.36)	9,037.00^b (579.40)
Net income (USD)	25,765.50	16,462.00	23,850.50	15,752.00	22,513.00	14,695.00	24,043.00^a (1,686.87)	15,636.33^b (1,290.50)

Note: Average values for 35 agroecological tea households and 31 conventional tea adopters. Different letters indicate difference at significance $P < 0.05$ level, according to the Tukey (HSD) tests. Standard deviation values are given in brackets. Soil conversion practice did not have a significant impact on economic indicators of tea production.

2.7.2 Soil physicochemical parameters and AMF colonization

Soil physicochemical properties (soil texture, soil pH, OM, available P and total N) and AMF colonization are presented in Table 6. Soils of the trial tea plantations were mainly clay loam in texture, with the proportions of sand and clay range from 30 to 40% and soil texture properties across the treatments did not show any significant differences, suggesting that soil types among the experimental plots were similar. Regarding the soil chemical properties, agroecological management practices resulted in significant increases of soil pH and organic matter contents, compared to the conventional tea management approaches, regardless of land use history. Highest soil pH (4.69 ± 0.3) was observed in agroecological converted soils, while the lowest pH (4.11 ± 0.19) was recorded in the conventional original plots, indicating that all tea plantation soils were strongly acidic. Average soil OM contents (%) in agroecological tea sites were greater than 3.0, compared with 2.32 and 2.30 of conventional original and conventional converted plots, respectively. By contrast, total nitrogen (%) was greater in conventional tea soils (0.37 and 0.30) compared to agroecological tea soils (0.22 and 0.23 for original and converted soils, respectively), while available P contents remained almost the same whatever the treatments (Table 2). The highest P availability content was found in agroecological original plantation soils (48.38 mg/ 100 g soil), while the lowest was observed in agroecological converted gardens (38 mg/ 100 g soil). Interestingly, soil conversion practices did not lead to any significant changes of the soil characteristics, regardless of the cultivation approaches.

In this study, roots of tea plants were colonized by native AMF, but the frequency (F) and intensity (M) varied greatly from 67- 98% and 10- 38%, respectively (Table 6). Tea root mycorrhization responded significantly to different tea management practices, regardless of converted or non-converted soils. Highest F was observed in the plantations that practiced both agroecological management and soil conversion, which accounted for 38%. This proportion was more than 3 times

higher than the lowest figure for tea root samples collected from conventional original farms. While the average proportion of AMF frequency of tea roots was close to 85%, the figure for AMF intensity was only approximately 26%.

The principal component analyses (PCA) of the soil physicochemical indicators and tea root mycorrhization parameters are presented in Fig. 11a and 11b. The first two axes together explained nearly 52% of the cumulative variability. The first axis (F1), which accounted for approximately 32% of the accumulated variability, was closely related to soil chemical indicators including OM, N total, and soil pH (0.610; -0.619 and 0.45, respectively). By contrast, soil texture (silt and clay) was strongly linked to the third axis (F3), which represented around 16% of the variation in the dataset. Root mycorrhizal F and M were strongly linked to the first axis (0.688 and 0.806, respectively), and significantly correlated to soil OM, soil pH and soil total N.

The PCA observation charts clearly show the positioning of the agroecological and conventional tea farms but was unable to distinguish between the converted and non-converted plantations. The observations were well-distributed along the F1 axis, indicating that tea management methods significantly impacted soil chemical properties such as soil pH, soil OM and total N, rather than the soil texture. Also, agroecological tea plantations were mainly distributed to the right side, suggesting a positive impact of the management practice on soil OM and AMF root colonization, but negatively link to soil total N. In contrast, the conventional tea farm observations were predominantly distributed to the left, meaning they have lower values of soil OM, soil OM and F and M values about root mycorrhization, but greater values of soil total N compared to the agroecological tea plantations (Fig. 11c and 11d).

Table 6. Soil physicochemical characteristics and AMF root colonization frequency (F%) and intensity (M%) of the tea plantations with different management practices and land use history

Plantations	Soil texture			Soil chemical characteristics				AMF colonization	
	Sand (%)	Silt (%)	Clay (%)	pH (H ₂ O)	OM (%)	Available P (mg/100g)	Total N (%)	F (%)	M (%)
AO	42.15 ^a (4.80)	19.57 ^a (2.28)	38.28 ^a (3.07)	4.52 ^{ab} (0.39)	3.08 ^a (0.21)	48.38 ^a (4.25)	0.22 ^b (0.09)	98.33 ^a (4.12)	34.95 ^a (3.45)
AC	41.18 ^a (5.71)	22.57 ^a (3.75)	36.25 ^a (4.25)	4.69 ^a (0.30)	3.04 ^a (0.30)	38 ^a (3.32)	0.23 ^{ab} (0.09)	97.46 ^a (5.06)	37.88 ^a (3.62)
CO	38.33 ^a (6.23)	20.27 ^a (3.96)	41.40 ^a (4.39)	4.11 ^b (0.19)	2.32 ^b (0.23)	39.14 ^a (3.24)	0.37 ^a (0.021)	66.95 ^c (7.59)	10.11 ^c (3.05)
CC	34.39 ^a (6.56)	24.87 ^a (3.90)	40.74 ^a (4.18)	4.23 ^b (0.28)	2.30 ^b (0.26)	42.33 ^a (3.12)	0.30 ^{ab} (0.018)	80.3 ^b (7.24)	20.51 ^b (4.17)

Note: F%: frequency of mycorrhization; M%: Intensity of mycorrhization. Average values for 25 and 45 samples per site group for soil characteristics and AMF assessment, respectively. For each variable, values followed by different letters are significantly different at $P < 0.05$ (pairwise comparisons of the means using the Tukey (HSD) tests). Standard deviation values are given in brackets. AO: Agroecological original; AC: Agroecological converted; CO: Conventional original and CC: Conventional converted.

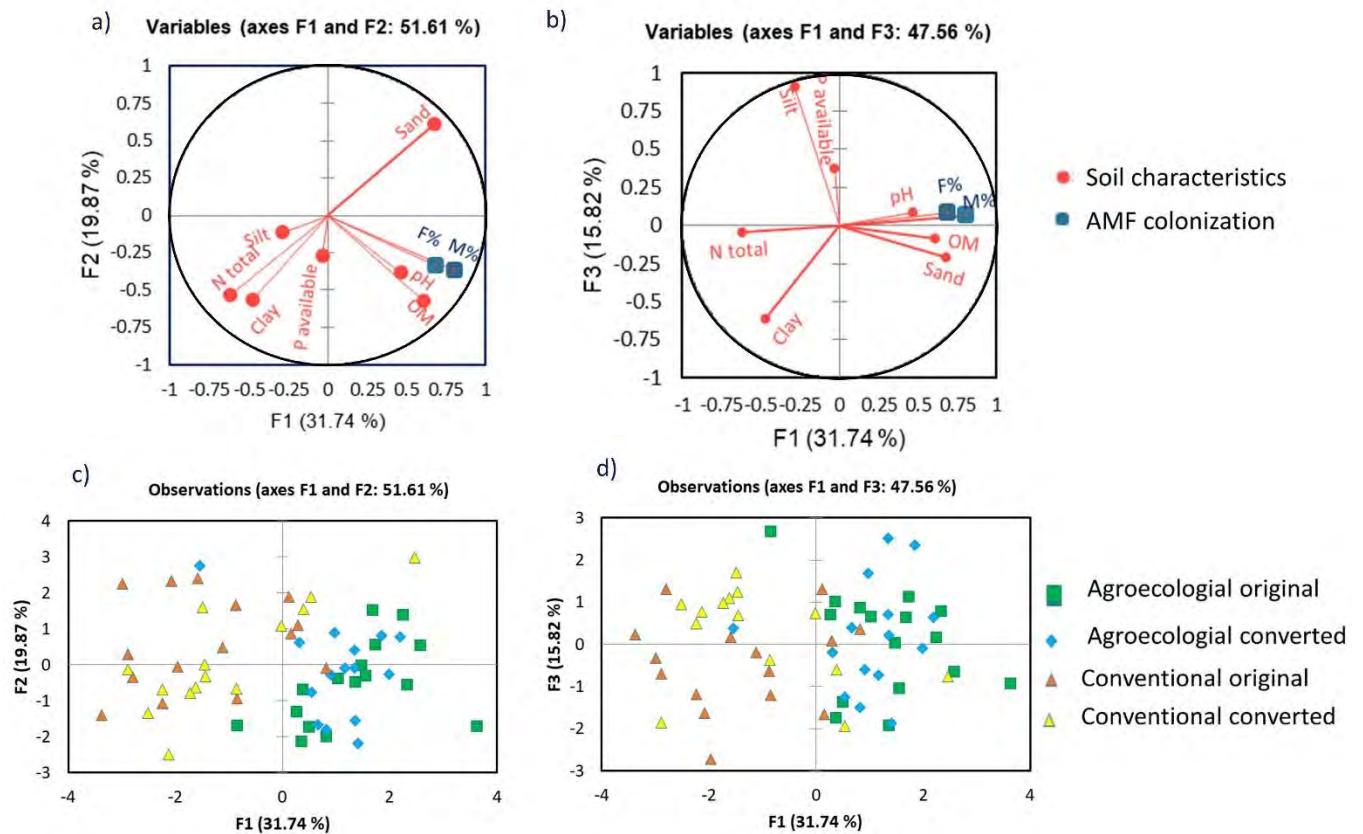


Figure 11. Principal component analysis (PCA) of soil characteristics and the AMF colonization of tea roots collected from agroecological and conventional tea plantations. a) and b): variable correlations with F1-F2 and F1-F3 axes, respectively. c) and d): sample ordinations along with F1-F2 and F1-F3, respectively, each point represents a single sample

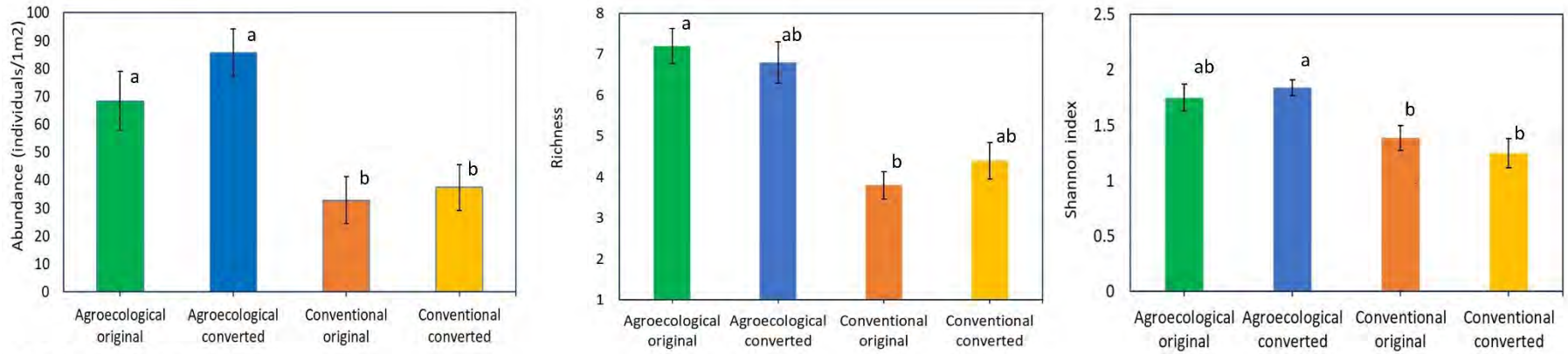
2.7.3 Soil fauna

Ecological indices of soil macro and mesofauna are presented in Fig. 12 and Table S4, S5 and S6 (Appendix 4). First, densities of soil fauna in agroecological original and agroecological converted were 68 and 86 individuals/m² respectively, while for conventional original and conventional converted treatments, the values were only 33 and 37 individuals/m², respectively. The abundance of soil mesofauna was strongly affected by management practices but was not always significantly different by extracting methods. With regards to the results obtained about mesofauna with the 2 different protocols, by centrifugation extraction we found 92, 129, 58 and 68 (ind./100 g fresh soil)

for agroecological original, agroecological converted, conventional original and conventional converted treatments, respectively, while the values extracted by employing funnel method were 80, 101, 58 and 68 (ind./100 g fresh soil), respectively. Community richness and Shannon index were also significantly different between the agroecological and conventional treatments ($P < 0.05$, Fig. 12 and Table S4, Appendix 4), but did not statistically differ between the extraction methods. For both soil mesofauna and macrofauna, the highest values of richness and Shannon index were recorded in agroecological converted and agroecological original treatments, which approximately doubled than the figures in conventional converted and conventional original treatments, regardless of the extraction methods. In contrast, soil conversion and its interaction with cultivation approach did not result in any significant difference of the soil fauna community indices and diversity index.

For soil fauna community composition, only 8 different soil fauna groups and 13 soil mesofauna groups were found in the experimental tea plots. Among the groups, earthworms were the dominant soil macrofauna species, accounting for nearly 34%, followed by centipedes and millipedes. Different tea cultivation methods also lead to a significant difference in the abundance of earthworm, centipede, spider and millipede species, while the impacts on other groups were not significant. For soil mesofauna, oribatei, millipede and enchytraeids were the most abundant groups, regardless of the extraction techniques. Interestingly, apart from millipedes, other mesofauna group intensities were not significantly affected by both cultivation and soil conversion practices (Table S5 and S6, Appendix 4).

Soil macrofauna



Soil mesofauna

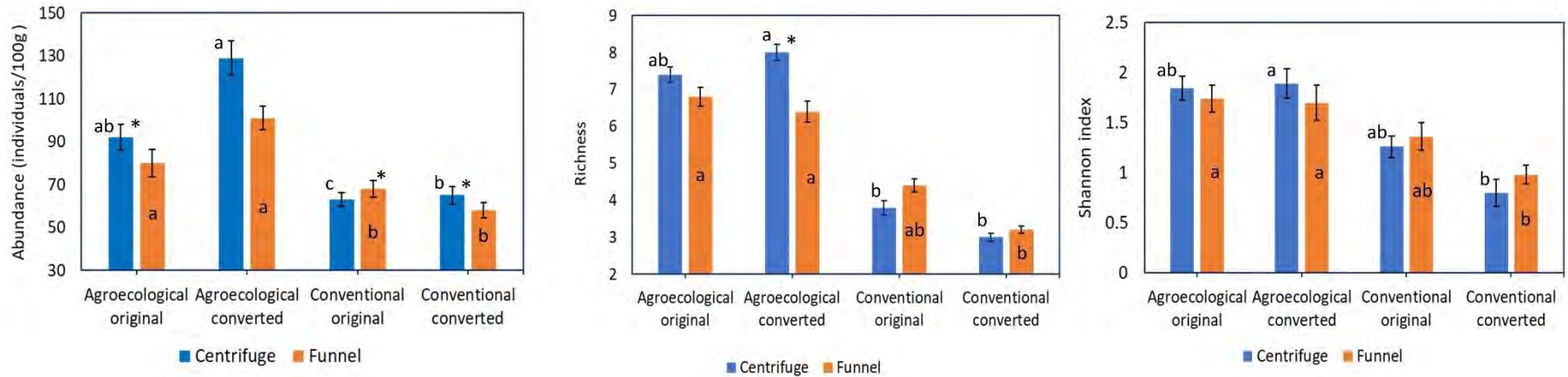


Figure 12. Variations in diversity indexes of the soil macrofauna (above) and mesofauna (below) observed in agroecological and conventional tea plantations. Average values for 10 samples per site group. Letters indicate difference in abundance (individuals/m² ± SD for soil macrofauna and individuals/100 g fresh soil ± SD for mesofauna), richness and Shannon diversity (mean ± SD) between management practices within extraction method at significance < 0.05 level, while (*) indicates the differences between mesofauna extraction methods within treatment at significance < 0.05 level

2.7.4 Tea yield, yield component and quality assessment

From 2019- 2021, tea yield and its components recorded in conventional tea plantations were consistently higher than those from agroecological plots, but these increases were not always significantly different (Table 7). Average tea yield ranged from around 14.1 tons to more than 16.3 tons year⁻¹ ha⁻¹, while average shoot density and weight of 100 shoots varied from nearly 580 to 700 (shoots/m²) and 31.8 to 36.6 (g), respectively. The conventional converted tea plantations produced the highest tea yield over the observation period, which accounted for 16.3, 16.0 and 15.9 (tons year⁻¹ ha⁻¹) for the years 2019, 2020 and 2021 on average, respectively, while the lowest yield was recorded in the agroecological original treatment over the observed period, which ranged from 14.19- 14.59 tons year⁻¹ ha⁻¹. Also, there was a reduction in tea yield and yield components in 2020 and 2021, compared to the figures in 2019. Over the 8 annual harvests, tea yield, number of shoots and shoot biomass were highest in the July and August/September harvests, which are summer times in the research areas, and then dropped quickly in the following harvests. The yield and shoot densities of tea harvested in the summer seasons were generally doubled that in the first (spring) and last yearly harvests (winter seasons) (Fig. 13).

Fig. 14 presents the sensory evaluation results of the green tea samples including dried tea leaf appearance, color, smell and taste of the tea infusion. Among the four attributes, the average marks for tea leaf appearance were significantly higher in conventional tea products (4.51 and 4.56 for conventional original and conventional converted tea leaves, respectively), compared to the agroecological dried tea (4.08 for agroecological original and 4.07 for agroecological converted tea leaves). In contrast, average marks given for smell and taste of agroecological tea infusion were significantly greater than for the conventional products. Agroecological original teas obtained the highest marks for both the brew aroma and taste, which amounted to 4.63 and 4.61 respectively, while the lowest marks were given to the conventional converted (4.15) and conventional original

(4.18). Conversely, there was no significant difference in the marks given for color of tea brew, which accounted for 4.5 on average. Overall, agroecological tea products obtained a significantly greater mark (≈ 18) compared to the tea products that were conventionally cultivated (≈ 17.3). As a result, all the green tea samples studied obtained the “Good” grade (total marks: 15.2- 18.1) (Fig. 14). As for the qualitative sensory description, all the dried tea leaves were young green, wiry, downy and creepy, even though the intensity of the creepiness and color appearance were different. Also, the color of converted and non-converted tea brew was qualitatively different, regardless of the management method. Infusions of tea samples harvested from non-converted farms were green and bright, while that of converted tea plantations were pale yellow-green, clear and medium body. The intensities of the fragrance and freshness (aroma) and sweetness after testing (taste) were also clearly different among agroecological and conventional tea products, which are crucial factors affecting the evaluation marks given to each type of infused tea (Fig. 14).

Table 7. Tea yield and yield components as affected by different tea cultivation methods (agroecological vs conventional) and land use history (converted and non-converted)

Plantations	2019			2020			2021		
	Shoot density	Shoot weight (100 shoots)	Yield (tons/ha)	Shoot density	Shoot weight (100 shoots)	Yield (tons/ha)	Shoot density	Shoot weight (100 shoots)	Yield (tons/ha)
AO	633 ^a (112)	31.8 ^b (2.95)	14.59 ^b (1.11)	577 ^b (31.58)	32.6 ^b (3.64)	14.32 ^a (0.85)	592 ^b (63.61)	32.1 ^b (1.51)	14.19 ^b (0.86)
AC	640 ^a (52)	33.2 ^b (3.11)	15.05 ^b (0.76)	584 ^b (62.61)	31.2 ^b (3.27)	14.64 ^a (0.68)	608 ^b (35.33)	33.5 ^b (1.50)	14.48 ^b (0.57)
CO	687 ^a (13.1)	35.8 ^a (3.11)	15.89 ^{ab} (0.95)	635 ^{ab} (63.94)	34.0 ^a (3.87)	15.51 ^a (0.74)	615 ^a (89.62)	33.9 ^{ab} (4.61)	15.33 ^{ab} (0.66)
CC	696 ^a (58)	36.6 ^a (2.66)	16.32 ^a (0.43)	647 ^a (108)	33.6 ^a (1.14)	16.04 ^a (0.50)	656 ^a (68.58)	34.3 ^a (2.91)	15.93 ^a (0.50)

Note: Shoot density (shoots / m²), shoot weight (weight of 100 tea shoots), yield (tons of fresh tea leaves ha⁻¹ year⁻¹). Average values for 15 samples per site group per year (shoot density and shoot weight) and 40 samples per site group per year (tea yield). For each variable, values followed by different letters are significantly different at P < 0.05, according to the Tukey (HSD) tests. Standard deviation values are given in brackets. AO: Agroecological original; AC: Agroecological converted; CO: Conventional original and CC: Conventional converted.

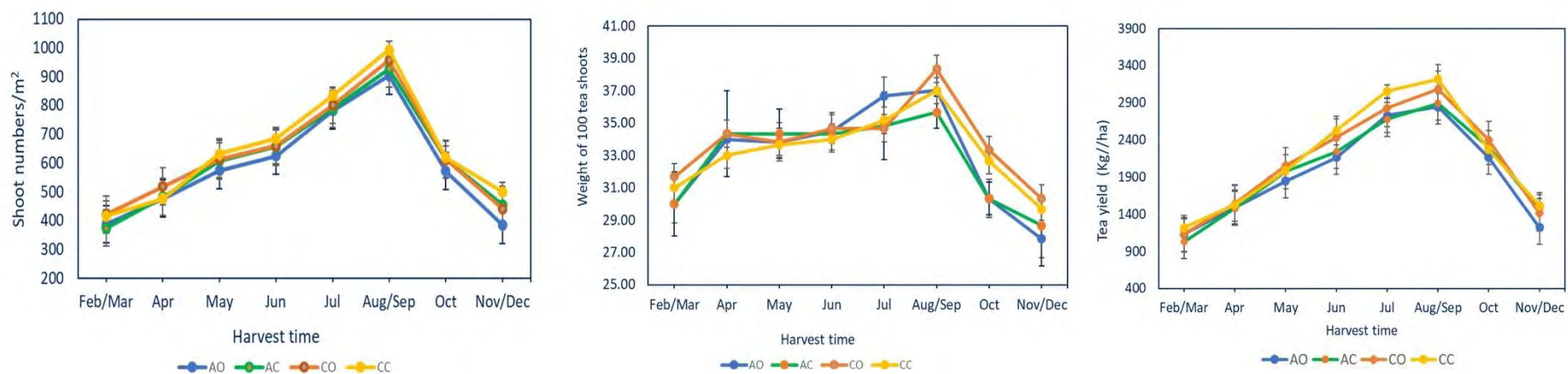


Figure 13. Tea crop yield and yield component changes over the yearly harvest times observed from 2019-2021 in agroecological and

conventional tea plantations. For tea shoot number and shoot weight, the means were based on 45 samples per site group, while the average yields were for 120 samples per site group. AO: Agroecological original; AC: Agroecological converted; CO: Conventional original and CC: Conventional converted

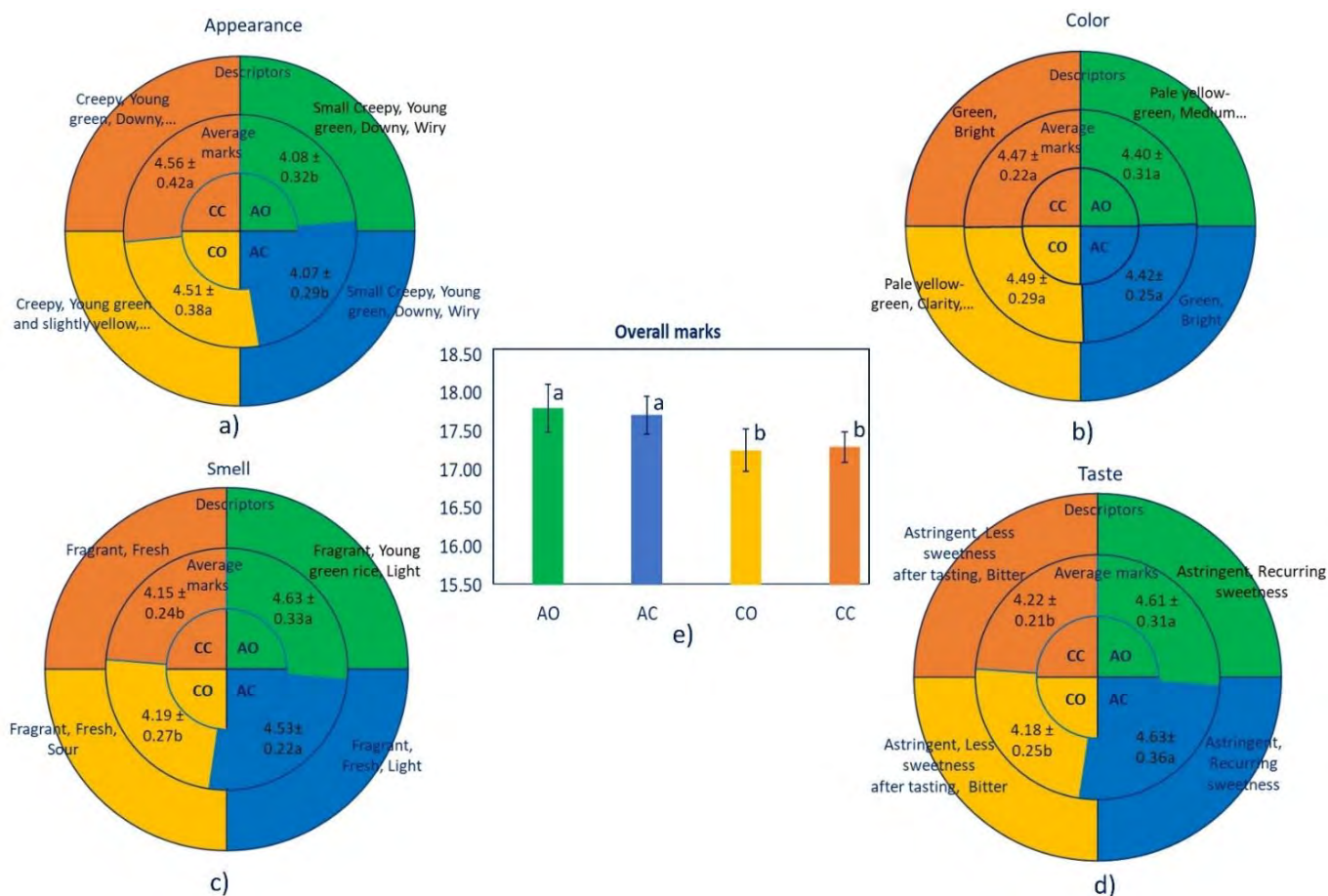


Figure 14. Sensory evaluation of green tea samples from agroecological and conventional tea plantations. a) Appearance; b) Color; c) Smell; d) Taste and e) Overall marks of the sensory properties. Sensory marks are given in average of 60 samples per site group with standard deviation values. Different letters indicate a significant difference at $P < 0.05$ (pairwise comparisons using the Tukey (HSD) test). AO: Agroecological original; AC: Agroecological converted; CO: Conventional original and CC: Conventional converted

2.8 Discussion

2.8.1 *Production economic efficiency*

Aside from the environmental advantage, economic benefit has been considered as one of the most important drivers for moving from conventional to agroecological and organic farming, not only for tea production (Bui and Nguyen 2020; Qiao et al. 2016; Viet San et al. 2021), but other cropping and livestock systems (Bouttes et al. 2019; Eyhorn et al. 2018). Our study shows that the agroecological tea farming provides a significantly greater net income for tea farmers compared to conventional tea management. This finding is similar to a number of studies (Deka and Goswami 2021; Doanh et al. 2018; Tran 2008) reporting that organic tea adopters earned a higher net income compared with the non-adopters, which mainly resulted from the premium price of organic tea products to offset the increased labour costs and yield reduction. Previous investigations also indicated that as new and more complex production systems, agroecological and organic farming required more capital investment than the conventional or traditional production systems, and it has been generally believed that only large scale farms could afford this (Azadi et al. 2011; Bui and Nguyen 2020). Our study confirmed this as the annual investments for labour, pesticides and organic fertilization and other maintenance costs for agroecological tea management method were significantly higher than those of conventional tea farmers. Instead of investing in chemical fertilizers and pesticides, agroecological tea growers need to spend more on alternatives such as organic fertilizers, biofertilizers, nanofertilizers and biopesticides, which are generally more expensive due to the technical complexity, limited availability and larger required amounts compared with the chemical inputs (Duran-Lara et al. 2020; Essiedu et al. 2020). Surprisingly, we observed that numerous small tea farms in the research region with a total area of less than 1,000 m² have been converted to practice organic and agroecological methods over the past 5 years. It is possible that a significant difference in selling price of agroecological tea products, along with the

subsidies from local governments and other agencies have encouraged tea growers to apply agroecological management practices (Doanh et al. 2018; Qiao et al. 2016). Recently, a growing concern regarding the harmful effects of agrochemicals on human health and the environment also plays a part in promoting tea farmers from converting their conventional tea to organic management practices (Doanh et al. 2018; Viet San et al. 2021).

2.8.2 Soil physicochemical properties and AMF colonization

Agroecology has long been known to benefit soil chemical and biological properties, while the negative impacts of conventional farming practices on soil health have been widely recognised (Cárceles Rodríguez et al. 2022; Gianinazzi et al. 2010). Our study showed that soil pH and OM content observed in agroecological tea plantations were significantly higher than the figures for the conventional tea plots, while total N was higher in conventional systems, which could be attributed to several mechanisms. First, intensive use of synthetic chemical fertilizers of conventional tea adopters, particularly nitrogen to ensure tea productivity, and as a replacement for soil fertility loss, has caused serious tea soil acidification due to the nitrification processes (Li et al. 2015; Viet San et al. 2022; Yan et al. 2020). We noted that conventional tea farmers in the studied region used up to 1,200 kg/ha/year of single nitrogen fertilizers (urea, ammonium nitrate) for ensuring high tea productivity and replacing soil nutrient loss, excluding the N amount from NPK compound annual applications. When an intensive amount of nitrogen fertilizer is applied, around 2,700 kg/ha/year, tea plants can only absorb around 18.2%, and the majority (up to 52%) will be stored in the tea soils, which can lead to an increase of soil nitrogen (Chen and Lin 2016; Xie et al. 2021). Also, tea plants take up the nutrient directly and an equivalent proton is subsequently excreted into the rhizosphere, causing hydrogen ion concentration to increase (Viet San et al. 2022; Yan et al. 2020). By contrast, agroecological tea growers employed organic and biofertilizers as the main soil nutrient supplies, which can restore soil pH due to their buffering capacity and higher pH values compared to

that in the tea acidic soils (Cornelissen et al. 2018; Gu et al. 2019; Ji et al. 2018). Increasing tea plantation age and plant density can also accelerate soil acidification, as tea roots could release organic and carbonic acids into the rhizosphere, decreasing soil pH (Hui et al. 2010; Viet San et al. 2022). Additionally, organic fertilizers and organic mulches that have been applied in the plantations such as fern (*Gleichenia linearis*), Acacia and Eucalyptus barks, rice straw and other plant residues supplemented a high input of organic materials into the tea soils, which can also increase tea soil organic carbon storage and organic matter (Cu and Thu 2014a; Li et al. 2014; Viet San et al. 2021). Tea plants prefer acidic soil with the optimal soil pH values from 4.5 to 5.5, but strongly acidic soils could lead to numerous consequences for tea growth and quality, such as nutrient leaching and imbalance, and heavy metal toxicity (Ni et al. 2018; Zhang et al. 2020). With regards to soil available P, our results are contrasted with Han et al. (2013) who indicated that available P concentrations were significantly different between organically and conventionally managed tea farms. This may be due to the inorganic (mainly NPK compounds) and organic fertilization by conventional and agroecological tea adopters in the region provided an equivalent amount of phosphorus for tea plantation soils. Supplying sufficient amount of phosphorus is essential for tea growth and productivity, as the vigorous growth of young tea trees, and frequent harvests of tea leaves require a large demand for P, thereby reducing the total P content of the tea plantation soils (De Schrijver et al. 2012; Wu et al. 2020b). Soil P availability also plays a key role in affecting the plant mycorrhization (Herrmann et al. 2016; Wang et al. 2020).

Arbuscular mycorrhizal fungi (AMF) has been widely known to be associated with a wide variety of plants and playing a key role in plant nutrition by providing access to soil-derived nutrients (Bhantana et al. 2021; Herrmann et al. 2016). AMF communities are affected by a number of environmental factors, such as soil characteristics, host plants and cultivation methods (Ji et al. 2022; Xu et al. 2017). In our study, the average AMF frequency (F) and intensity (M) of the

agroecological tea roots were significantly greater than in conventionally managed tea plantations. This finding is similar to observations made by Wu et al. (2020b) who indicated that organic tea management significantly increased tea soil AMF contents, while Wang et al. (2017) revealed that long-term application of chicken manure strongly modified tea soil fungal communities. Singh et al. (2008) also showed that the average AMF frequency of roots collected from natural and cultivated tea plantations was 77.6% and 86.5%, and intensity was 11.3 and 23.9%, respectively. Likewise, stimulation of AMF growth by incorporation of different organic amendments such as rice straw and organic compost has been widely reported in other cultivars (Hammer et al. 2011; Qin et al. 2015). In contrast, numerous studies indicated that mineral fertilizers, especially N and P, adversely affected AMF growth in tea plantations (Toman and Jha 2011; Wu et al. 2020b), in arable soil (Lin et al. 2012a) and in rotation system (Qin et al. 2015). It was reported that AMF prefer a near neutral or alkaline soil pH for optimal growth and are strongly correlated with phosphorus level in soil, therefore, intensive application of mineral fertilizers changed the soil pH and P volume in the rhizosphere, thus affecting AMF communities (Helgason and Fitter 2009; Ma et al. 2021). Furthermore, we observed that the availability of P in this study was negatively correlated with tea root AMF frequency and intensity, suggesting that tea plants may find the necessary elements in the soil and thus the symbiosis with AMF was less profitable (Herrmann et al. 2016; Van Geel et al. 2016).

In our study, tea root AMF frequency and intensity observed in converted tea soils were significantly higher than in original tea plantation soils. These findings are consistent with previous investigations which have reported that land use history significantly affected soil fungal communities, which could be attributed mainly to alteration in soil environmental factors, in which soil pH is a proxy (Monkai et al. 2018; Wu et al. 2020b; Zheng et al. 2020). Since the highest root mycorrhizal intensity was only 38% across all the trials, it suggests that other options such as

application of bioinoculants containing effective AMF should be introduced to improve tea root mycorrhization, and subsequently soil health and plant growth (Bag et al. 2022; Shao et al. 2018). It has been reported that AMF's incorporation significantly enhanced soil accessible P concentrations and encouraged P absorption by tea plant, as well as improved tea growth characters (root biomass, plant height) and quality indicators such as amino acids, polyphenolic compounds, caffeine, total protein content, and sugars (Cao et al. 2021; Mei et al. 2019; Shao et al. 2021).

2.8.3 Soil macro and mesofauna

Intensive agriculture is known to have long lasting and negative effects in soil biota, making soil food webs less diverse and composed of smaller bodied organisms (Liiri et al. 2012; Tsiafouli et al. 2015). In this study, the abundance, richness and Shanon index of soil macro and mesofauna were significantly greater in agroecological treatments compared to those of conventional tea plots (Table S5, S6 and Fig. 3). However, compared to previous studies of soil faunal communities in tea and other cropping systems, these indices are significantly lower. For instance, a world-wide investigation conducted in 41 countries indicated that soil macroinvertebrate abundance in cropping systems ranged from 232 to 867 individuals/m² (Lavelle et al. 2022). Yu et al. (2021) also found up to 26 different soil faunal groups in tea cultivars, with the Shannon index value of 4.65. In our study, the number of soil macrofauna individuals/ m² was only from 37 to 86, and only 8 groups of soil fauna occurred in tea plantations, regardless of the tea soil management practices. Strongly acidic soils could be one of the key factors that negatively affect soil faunal communities. For example, it was reported by Senapati et al. (2002) that in tea plantations, a low soil pH (pH < 4) could lead to a loss of up to 70% of soil biota. Greater abundance of soil fauna communities of organic and agroecological farming over its conventional counterparts have been widely reported (Domínguez et al. 2014; Sofu et al. 2020). Manure and organic mulching applications have been widely recognized to positively affect soil faunal communities and functional structures, since these practices not only

provided readily available food sources, but also regulated soil temperature and moisture (Jiang et al. 2021; Wang et al. 2016a). Particularly, Murray et al. (2006) found that organic fertilization directly supplied detritus and indirectly modified soil nutrient environment for fauna, which subsequently induced an increase of soil faunal abundance. In contrast, conventional agriculture consistently has negative impacts on soil biota, which could be attributed to detrimental effects of intensive agrochemical inputs, monocropping that systematically simplify soil food web diversity, and microclimate modification due to residue removals. Likewise, Domínguez et al. (2014) suggested that non-use of agrochemicals would be enough to produce shifts in soil faunal diversity.

Several studies have also examined the effect of different extraction methods on diversity indices and communities of soil fauna. Active methods such as the Baermann funnel and passive approaches such as filtering and flotation-centrifugation are among the most recognized practices for sampling and extracting soil fauna, which based on different physicochemical principles of soil fauna (Domingo-Quero and Alonso-Zarazaga 2010). Dritsoulas and Duncan (2020) indicated that passive extraction methods consistently recovered significantly more soil microarthropods compared to the active techniques. This is in accordance with our findings since the ecological indices (abundance, richness and Shannon index) derived from the centrifugation method were constantly greater than the figures for the funnel techniques, though the differences were not always significant (Fig. 3). In addition, the present study results on soil fauna composition are consistent with some previous studies, which indicated that earthworm is the dominant soil macrofauna group in tea plantations (Jamatia and Chaudhuri 2017b; Kahneh et al. 2022).

2.8.4 Tea yield, yield components and green tea sensory quality

Organic and agroecological farming typically have lower harvest yields in comparison to conventional agriculture, which has raised concerns about the potential role of these farming

methods as a sustainable strategy in meeting the increasing demand for food and other agricultural services (Schrama et al. 2018; Seufert et al. 2012). Several studies have reported tea harvest yield gaps between conventional and organic tea farming systems (Deka and Goswami 2021; Doanh et al. 2018; Qiao et al. 2016). This is consistent with our findings since agroecological tea adoption consistently produced less tea harvest yield than the conventional tea implementation over the 3 years of observations (differences were not always significant -Table 3). Agroecological and organic tea farming systems rely on non- chemical inputs such as organic materials and biofertilizers for maintaining crop productivity, while restoring soil health and mitigating environmental pollution (Gui et al. 2021; Viet San et al. 2021). In return, these resources may not provide enough sufficient macro and micro nutrients, such as nitrogen and phosphorus for crops to growth and obtain as high yields as conventional method that employs intensive application of synthetic fertilizers, especially during the transition period (the first 3-5 years since the conversion from conventional to organic farming management) (Doanh et al. 2018; Han et al. 2018). A comprehensive investigation by Seufert et al. (2012) also revealed that the yield gap between conventional and organic farming systems could be up to 34%, depending on conditions such as site characteristics, crop types and level of intensification. Han et al. (2018) also concluded that tea yields in organically managed agro-ecosystems are generally 8–20% lower compared to conventional systems. However, our observations in 66 different tea plantations from 2019- 2021 showed that the yield difference between conventional and agroecological tea systems was less than 8% on average (Table 1). In the studied regions, the agroecological tea adopters invested heavily in organic fertilizers, biofertilizers, organic mulches, and other organic materials such as soybean or fish powder, to replace mineral fertilizers, as well as labour costs for weed, pest and disease managements, all of practices positively contributed to increased tea yield. In addition, the difference in the duration taken from conventional to agroecological farming could play a significant part in reducing the yield gap

between conventional and agroecological farming systems, since longer application duration would lead to positive changes in abiotic and biotic soil properties leading to a more efficient, spatially and temporarily stable farming system (Schrama et al. 2018).

Our findings about the tea leaf appearance are in line with the study by Luyen et al. (2014) who indicated that green tea leaves harvested in Tan Cuong commune, Thai Nguyen province were greener, less leafy, wirier and more creepy than tea leaves produced from other regions of the country, which were mainly attributed to the differences in geography, climate, cultivation practices and processing method. Also, the fragrance, fresh and light smell of the brewed teas, intensity of the astringence, sweetness and bitterness in the taste found in the present study were similar to previous reports concerning the sensory attributes of green tea (Luyen et al. 2014; Tang et al. 2020). Previous studies have indicated that the smell and taste of the green tea are mainly driven by the plant chemical components, such as the tea polyphenol with bitter taste and the astringency, while the sweet, umami taste of green tea generally originates from amino acids, especially theanine, which accounts for about 65 % of the total amino acid content in tea leaves (Pongsuwan et al. 2008; Tang et al. 2020). Finally, cultivation practices such as application of cow manure could alter the metabolism of amino acid, sugar and fatty acids in tea shoots, thus enhancing the human sensory preference for tea brewed aroma and taste (Sun et al. 2021). This correlated with our results that agroecological tea management practices which employed organic manure as the main nutrient supply, provide significant difference of sensory marks for tea products. Since the aroma and taste of tea products are key factors determining the quality grade of tea and its market price (Qin et al. 2013; Su et al. 2021), a significant increase in these quality indicators as results of organic tea management practices would enhance economic benefits for the adopters. Sumi and Kabir (2018) reported that the taste, natural content, and the nutrient value of organic tea makes it a popular choice for health-conscious customers. Qiao et al. (2016) also indicated that organic tea produced in

Wuyuan, China fetches a premium price and consistent purchase orders for organic tea products have been offered, providing stability and incentives for tea farmers for adopting and expanding organic tea production.

2.9 Conclusion

Soil acidification is becoming an increasingly severe problem in many tea growing countries, resulting in serious impacts on soil chemical properties, tea productivity and quality and the environment. To date, however, how low pH affects tea soil biological and physical properties as well as its management cost have been poorly explored. Agriculture wastes and products have demonstrated a great potential to mitigate soil acidification by tea cultivation and improve tea soil health. Being naturally alkaline with high pH value and buffering capacity, these materials could supply alkaline matter and essential elements to neutralize soil acidity and alter soil properties, positively influencing soil nutrient availability, enrich soil organisms and ultimately improve tea yield and quality indicators. While promising, their expanded uses would need further understanding to improve their application efficacy while reducing any potential negative consequences on the environment. In addition, the risks of introduction of heavy metal and pathogens from animal manures, compost and biochar applications have been widely reported (Alegbeleye and Sant'Ana 2020; Dai et al. 2017), but how they could affect soil and tea plants have not been clearly understood. Moreover, most of reports on effective impacts of biochar for correcting soil acidification have been the outcomes of laboratory or glasshouse studies, thus the results need to be validated in field conditions (Dai et al. 2017). Finally, the majority of studies on utilizing agricultural wastes in tea cultivation to date have been implemented in China, with specific but limited soil characteristics, climate conditions and tea management practices. It has been clearly indicated that differences in such conditions could significantly affect the effectiveness of these soil

acidification ameliorants (Gu et al. 2019; Siedt et al. 2020; Wu et al. 2020a). This research gap highlights the need and opportunities for further investigations in other systems to provide comprehensive knowledge and reliability in recycling these soil amendments.

This comprehensive field study also compared the impacts of agroecological and conventional tea management practices on soil health properties, tea productivity, economical benefit and quality in Thai Nguyen province as well as in Vietnam. We show that converting conventional tea adoption to agroecological management practices significantly increased tea root AMF intensity by up to 24%, soil macro and mesofauna by 110% and 60%, respectively. Organic fertilizers and manure incorporations also significantly reduced soil acidification rates due to their naturally alkaline characteristics, provided supplement organic matters, thus improving soil OM, AMF colonization and soil faunal abundance and diversity. In contrast, soil conversion from paddy and other annual crop fields to tea plantations did not lead to any significantly adverse effects on soil health properties, suggesting that cultivating tea in the newly established tea lands could be as effective as cultivating tea in the long-term tea plantation lands. Despite the lower tea yields, agroecological management method led to a significant increase in net income for tea farmers, which was mainly driven by premium price of agroecological tea products and other credits from supporting agencies. These practices, therefore, could be scaled up in Northern Vietnam and other regions which share similar natural and socioeconomic conditions for a more environmentally sustainable economic tea production.

3. CHAPTER 3: Response of soil biodiversity and crop productivity to liming in tea plantations in Northern Vietnam

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3.1 Abstract

In Thai Nguyen province, farmers convert a significant part of the paddy rice fields into tea plantations to get more outcomes, especially with organic tea. However, soil pH in tea plantations is usually strongly acidic and farmers widely apply lime to control tea soil acidification and avoid toxicity effects on the plant growth and tea yield. However, the impact of these strategies on soil biodiversity and crop productivity in tea farming remains poorly investigated so far. Here, we assessed the impacts of liming on soil chemical properties, soil and organic mulch macrofauna, soil microbial communities using the rDNA gene and ITS gene sequencing analyses, and tea yield on both converted and non-converted tea plantation soils, i.e., soils used for tea plantations for more than 20 years versus former paddy land recently converted to tea plantations. We showed that 9 months after application, liming significantly enhanced soil pH by 0.4 units, soil organic matter (OM) content by 0.28%, and P availability by 23.1 mg/100g, while it strongly reduced soil exchangeable Al and Mn by 2.52 and 0.25 (cmol/kg), respectively. Compared with non-converted tea plantation soils, the means of soil pH, OM, P and Al availability observed in tea soils that were converted from paddy fields were significantly greater. Macrofaunal abundance and community composition recorded in soil and organic mulch were significantly altered by lime addition but were not significantly correlated to land types. Likewise, liming significantly enhanced tea root mycorrhization by arbuscular mycorrhizal fungi (AMF), both in intensity and frequency, as well as tea yield, irrespective of land types. In contrast, the relative abundance and composition of soil bacteria, fungi, and AMF showed a significant response to land types and to the interaction between liming and land type, while the impact of liming alone was not significant. This chapter provides a better understanding of how liming and land use history affect tea soil food webs, plant growth and tea yields. Liming emerges as a highly effective strategy to manage soil acidification, to restore soil biodiversity and to enhance crop productivity in this tropical region.

3.2 Introduction

Vietnam is ranked as the 7th and 5th worldwide in terms of tea production and export, being capable of producing more than 1 million tons of fresh tea leaves annually, which mainly come from small household farmers. Since 2010, the tea industry has employed around 1,5 million people per year (Van Ho et al. 2019; Viet San et al. 2021). Over the last decade, the country has encountered an alarming soil health degradation problem resulting from long-term intensive tea cultivation which has caused severe soil acidification, soil nutrient loss and environmental pollution (Viet San et al. 2023). This problem results mainly from inappropriate management practices and the natural accumulation of acid excreted by tea plants, causing soil biodiversity loss, nutrient imbalance and leaching and subsequently reducing tea growth and quality (Viet San et al. 2022; Yan et al. 2020). To address this major issue, farmers apply commercial lime to reduce soil acidification, and to provide nutrients such as Calcium. Soil pH is known as one of the key drivers for soil biological diversity (Goulding 2016; Holland et al. 2018), but so far, we have no information about how lime application affects soil biodiversity in tea plantations in Vietnam.

Previous studies conducted in agroecosystems (croplands and grasslands) and natural ecosystems such as forests reported the benefits of lime amendment on several soil characteristics. Numerous investigations (Holland et al. 2018; Mahmud and Chong 2022; Tunney et al. 2010) reported that liming decreases Al toxicity, increases soil pH and phosphorus (P) and magnesium (Mg) availability, and improves soil physical conditions and structure. Meanwhile, Abdalla et al. (2022) and Keiblinger and Kral (2018) showed that liming can mitigate soil degradation by its buffering capacity on soil pH. In terms of soil microbial properties, liming can significantly affect both bacterial abundance and community structure (Ma et al. 2018; Yin et al. 2021b), fungal community composition and diversity (Wan et al. 2019; Wang et al. 2021), especially soil arbuscular

mycorrhizal fungi (AMF) diversity and root AMF infection (Guo et al. 2012; Holland et al. 2018). All these organisms are known to contribute to plant performance and crop yields.

Recent work reported that liming effects can vary significantly with geographic location, soil depth, host crops and the type of management practices (Abdalla et al. 2022; Holland et al. 2018). The context-dependency of liming effect on soil properties needs to be addressed in the specific context of Vietnam where land use change is very common. In the country, the conversion from natural lands into agricultural lands, and from annual croplands to perennial- based farming systems could subsequently lead to direct effects on soil health-related properties, as it involves different vegetation cover and soil management practices (Dawoe et al. 2014; Graham et al. 2021; Merloti et al. 2019). Currently, there is still a lack of knowledge of the effects of liming on tea soil biodiversity and yield through improving soil health-related properties (Li et al. 2022; Wang et al. 2018a; Yin et al. 2021a) and how these effects are impeded by different land types in tea plantations in Vietnam. To fill this gap, our study aimed to quantify the effects of liming under converted and non-converted soils on the response of different soil components; soil chemical characteristics, soil macrofauna, root AMF and soil microbial communities (AMF, fungi and bacteria) and on plant productivity indicators (yield, tea shoot density and shoot weight) in tea plantations in Northern Vietnam. We expected that lime incorporation significantly reduces soil acidity and affects tea soil chemical properties, driving significant alterations in macrofauna communities, tea root AMF colonization, soil microbial community diversity and composition, as well as tea yield indicators.

3.3 Material and Method

3.3.1 Site description and lime application

We selected 6 tea plantations (Tea variety was LDP1, all plantations were 9 years old, as of 2022) that had lime applied in January 2021 in Tan Cuong commune (21°32' N 105°45' E), Thai Nguyen

city, Thai Nguyen province, the largest tea producing province in Northern Vietnam. All of these agroecological farms have been investigated previously by comparison with conventional farms in Viet San et al. (2023). The mean annual temperature and precipitation in the region are around 23⁰C and 2250 mm, respectively, and the peak rainy season is from May to September (Xuan et al. 2013). Land areas used for tea production are generally slightly sloping (< 15°) and soil type is classified as Acrisols (FAO 1998) with a texture of 41.6% sand, 21% silt, and 37.4% clay. All the trial tea plantations have been under agroecological management practices for at least 6 years (by 2021), i.e., they received organic manure (chicken, cow and/or buffalo compost, 3 tons/ha/year) and commercial organic fertilizers (Tien Nong: OM 23 %, humic acid 2.5 %, total N 2.5 %; pH 5; moisture 20 %, 3 tons/ha/year), organic mulching (Fern, crop straw and Acacia tree barks), and integrated pest/ disease management (IPM/IDM). Of the 6 agroecological tea plots, three were original land for tea production, which referred to hill soil that have been used for tea plantations for at least 2 generations (20-30 years), never been flooded and slightly sloping (10-15°). The remaining three plots were converted lands, which were previously used as paddy fields and were converted to tea plantation soils for less than 10 years by adding hill soils with a depth of 0.8 -1.5 m on the top. Converted tea soils were used for flood irrigation and are less sloping (< 8°). Since 2017, these plantations have been in transition to organic tea production, where no chemical pesticides and fertilizers have been used to comply with the certification requirement. More details of these tea plantations have been described in Viet San et al. (2023).

The calcitic lime (CaCO₃ powder with 85% effectiveness) was purchased locally and applied in January 2021. Lime was applied at 1.5 tons/ha in the pruning season, at the inter-rows of tea plantations by removing the organic mulching materials on the soil surface then spreading powdered lime evenly on the designed soil surface and then replacing the mulching materials that were removed. Each trial plot was divided into two parts, including liming treatment plot with the area of

400 m², and the control (ranged from 800 to 1,600 m², depending on the total areas of the experimental fields). After liming, soil samples were collected monthly to monitor soil pH changes.

3.3.2 Soil and root sampling

Soil and root sampling was conducted in October 2021, 9 months after lime application. In the center of each liming and control plot, a sampling area of 60 m² (5 m x 12 m) was located, and 8 samples per plot were identified in the inter-rows, distanced by 5 m in width and 4 m in length, 20-30 cm away from tea trunks. Firstly, macrofauna (classified by the body diameter between 2 and 20 mm, as described by Gongalsky Gongalsky (2021), Ruiz-Lupi3n et al. (2022), was harvested by hands separately from organic mulch (20 cm x 20 cm) and soils (20 x 20 x 20 cm monoliths) in the morning to avoid effect of heat from the sun and other activities such as tea harvesting and irrigation. The macrofauna was then preserved in 70% alcohol and stored at room temperature until being identified at the group levels. Following the soil macrofauna sampling, 8 soil samples per treatment plot (\approx 15 g per sample) were collected using a spade and stored in 15 ml plastic tubes in cool boxes, then transferred to -80 3C for soil microbial analysis, and another 8 soil samples per plot (500 g each) were collected into large size resealable plastic bag, air dried and stored in room temperature for chemical assessment. Simultaneously, 8 finest tea root samples (\approx 35 g per sample) were collected by digging into soil areas within a 1 m circle from the same points for organic mulch and soil sampling then contained in paper envelopes and air dried for AMF analyses.

3.3.3 Soil chemical analyses

Soil chemical analysis consisted of soil pH (H₂O) (1/5, soil/water), soil organic matter (OM) (Walkley and Black 1934), phosphorus availability (Olsen P) (Olsen and Sommers 1982), total nitrogen (N) (Kjeldahl method, as described by S3ez-Plaza et al. (2013)). Soil exchangeable aluminum (Al³⁺) was extracted using 1 mol L⁻¹ KCL (Yerima et al. 2020), and exchangeable

manganese (Mn^{2+}) was extracted with $1 \text{ mol L}^{-1} \text{ CH}_3\text{COONH}_4$ ($\text{pH} = 7$), then determined by atomic absorption spectrophotometry (AAS) (Jaremko and Kalembasa 2014).

3.3.4 Tea root AMF colonization assessment

To determine tea root colonization, fine root samples were oven-dried at 40°C , and the ink and vinegar method was used for AMF staining (Vierheilig et al. 1998). Tea root AMF frequency (F%) and intensity (M%) were assessed by observing the presence and scoring the intensity (from 0-5) of fifteen root fragments, as described by Trouvelot et al. (1986).

3.3.5 DNA extraction and sequencing

Genomic DNA was extracted from 0.5 g of soils using the MP116004-500 Fast DNA SPIN kit for soil (MP Biomedical, Santa Anna, CA), according to Tournier et al. (2015). PCR amplification was done using the primer pairs 515F (5'- GTGYCAGCMGCCGCGGTAA-3')/806RB(5'- GGACTACNVGGGTWTCTAAT-3'), ITS1F (5'- CTTGGTCATTTAGAGGAAGTAA-3')/ITS2R (5' -GCTGCGTTCTTCATCGATGC-3') and AML1F (5'- CAGCCGCGGTAATTCCAGCT-3')/AML2R (5' -GAACCCAAACACTTTGGTTTCC-3') to amplify the fragments of the 16S rRNA gene and ITS region for the bacterial, fungal and soil AMF communities, respectively, and a barcode was included on the forward primers (Bahram et al. 2022; Herrmann et al. 2019). For bacterial sequences, PCR amplification was undertaken under the following thermal conditions: 95°C for 3 minutes, followed by 25 cycles of 95°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, while that for fungal DNA sequences were 95°C for 5 minutes, followed by 30 cycles of 95°C for 30 seconds, 53°C for 40 seconds a 72°C for 1 minute. Thermal cycling conditions for AMF samples were as follows: 95°C for 5 minutes, followed by 15 cycles of 95°C for 30 seconds, 50°C for 40 seconds and 72°C for 1 minute. Additionally, a final elongation step at 72°C for 5 minutes was performed for both bacterial, fungal and AMF samples, and after amplification, PCR

products are checked in 2% agarose gel to determine the success of amplification and the relative intensity of bands. Samples were pooled together in equal DNA concentrations and purified using calibrated Ampure XP beads. Afterwards, purified samples were used to prepare illumina DNA libraries. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on a MiSeq instrument following the manufacturer's guidelines.

3.3.6 Sequencing data analysis

Qiime2 (v.2017.6) was employed to denoise sequence and define operational taxonomic units (OTUs) (Andreo-Jimenez et al. 2021; Estaki et al. 2020). Raw sequencing data of soil bacteria, fungi and AMF was uploaded into Qiime2 with their associated quality scores using the artifact plugin. To reduce data size and thus enable the subsequent analyses, soil bacterial sequence reads were subsampled, and 50% of the bacterial dataset was retained. Demultiplexed data then were denoised with DADA2 plugin, and sequences (5'-3' and 3'-5') were joined and barcodes were removed. Sequences were retained only if they carried the correct primer sequences and were ≥ 200 bp long, according to visual inspection of the read quality. OTUs were defined by clustering at 99% similarity threshold, and singletons and chimeras were discarded (Dowd et al. 2008). Alpha and beta diversity analyses were conducted using the QIIME2 diversity plugin, and sequencing depth per sample was rarefied to the lowest number of observations (96,575; 12,159 and 9,695 reads for bacterial, fungal and AMF datasets, respectively) for subsequent analyses. Final OTUs were taxonomically assigned using BLASTn against a curated database derived from NCBI for bacterial taxonomy, (<http://rdp.cme.msu.edu>), UNITE for fungal taxonomy (<https://unite.ut.ee/repository.php>) and MAARJAM for soil AMF taxonomy identification (<https://maarjam.ut.ee/>).

3.3.7 Tea yield and yield component assessment

Tea yield and yield components in the different treatments were compared for 2 years, from 2021-2022. Tea yield components were measured as the density of tea shoots/m² and average weight of 100 tea shoots. To do so, a quadrat (1 m x 1 m) was randomly placed at the center of each trial plot during the harvest days, and all tea shoots presenting in the quadrats were manually picked, with 5 replicates per plantation. All harvested tea shoots (1 bud and 2 leaves) were then counted to assess the density, and 100 tea shoots were randomly selected for assessing their median weight. To measure the average tea yield (tons of fresh leaves/ha/year), all fresh tea shoots harvested from the treatments from 2021-2022 were weighted. For more information about how tea yield and yield components in the region were assessed, please refer to our previous publication (Viet San et al. 2023).

3.3.8 Statistical analysis

Microsoft® Excel, XLSTAT (Addinsoft 2016) and R software (R version 4.2.3, R Core Team (2022)) were employed for data analysis. Soil chemical and tea yield data were ln(x) transformed where needed to meet the normal distribution verification before two-way Analysis of Variance (ANOVA), and Tukey-HSD tests were performed for level differences within each factor (soil pH, OM, Olsen P, total N, Al³⁺ and Mn²⁺, tea yield and yield components). Differences in soil and mulch macrofauna diversity indexes (abundance, richness and Shannon index) were performed using the decostand () function in the Vegan package and then tested using one-way ANOVA, and community composition correlation and similarity were performed using the Principal component analysis (PCA) and Permutational Analysis of Variance (PERMANOVA, adonis() function). For soil microbial community assessment, the Phyloseq() function was employed to clean unidentified

OTUs, and all the OTUs with a relative abundance < 1% of the total reads of all samples were removed. Afterwards, soil microbial communities associated with different treatments were tested using the PERMANOVA test (number of permutations = 9999) followed by the pairwise comparison Tukey test, and the Nonmetric multidimensional scaling (NMDS) with the metaMDS() function were performed to visualize bacterial, fungal and soil AMF community dissimilarity among soil samples. Statistical test was considered significant at $P < 0.05$.

3.4 Results

3.4.1 Soil chemical characteristics

Nine months following treatment application, we found that liming has a positive and significant effect on soil pH, and soil Olsen P, but a negative impact on soil exchangeable Al^{3+} and Mn^{2+} . Newly established tea soils also significantly enhanced soil pH and soil OM, but also increased soil exchangeable Al^{3+} and Olsen P (Table 8 and Table S7), compared to the well - established tea plantation soils.

Table 8. Effect of lime application and land type on soil chemical characteristics of the tea plantations (mean \pm SD). Average values for 24 samples per site group.

Treatment/Factors	pH (H ₂ O)	OM (%)	Olsen P (mg/100g)	Total N (%)	Al ³⁺ (cmol/kg)	Mn ²⁺ (cmol/kg)
Original control	4.60 \pm 0.31d	3.20 \pm 0.35b	54.60 \pm 6.85b	0.21 \pm 0.08a	2.22 \pm 0.26bc	0.52 \pm 0.07a
Original lime	5.02 \pm 0.34b	3.42 \pm 0.36ab	65.64 \pm 7.15ab	0.27 \pm 0.06a	1.72 \pm 0.22c	0.42 \pm 0.05b
Converted control	4.86 \pm 0.21c	3.29 \pm 0.41ab	60.80 \pm 6.24ab	0.25 \pm 0.08a	5.60 \pm 0.48a	0.54 \pm 0.08a
Converted lime	5.18 \pm 0.26a	3.63 \pm 0.32a	72.86 \pm 7.62a	0.33 \pm 0.07a	3.58 \pm 0.34b	0.39 \pm 0.06b

Note: For each variable, values followed by different letters are significantly different at $P < 0.05$, according to the Tukey (HSD) tests

The highest soil pH (5.18 ± 0.26) was recorded in the converted-lime soils, while the lowest pH (4.6 ± 0.31) was found in the original tea soil without lime addition. Soil Olsen P ranged from 54.60 mg/100g (± 5.85 , original control soils) to 72.86 mg/100g (± 8.24 , converted limed plots), and soil OM content was also highest in converted lime plots (3.63 ± 0.32), followed by original limed fields (3.2 ± 0.35). By contrast, lowest soil Al^{3+} and Mn^{2+} were observed in original lime-treated soils, which were 1.72 cmol/kg (± 0.22) and 0.39 cmol/kg (± 0.06), respectively, while the highest Al^{3+} and Mn^{2+} levels (5.60 ± 0.48 cmol/kg and 0.54 ± 0.08 cmol/kg, respectively) were recorded in the converted control tea soils (Table 8). Converted lime plots also had the highest N contents (0.33 ± 0.07), while that of soil exchangeable Al^{3+} significantly reduced compared to unlime plots. Our joint Principal component analysis (PCA) also indicated that soil Olsen P is significantly correlated to soil pH and soil Al^{3+} , while the correlation between soil total N and OM is also significant and positive (Table 8 and Table S7).

3.4.2 Macrofauna

Lime application had a significant positive effect ($P < 0.05$, ANOVA test) on soil macrofauna abundance, richness and Shannon index, irrespective of land use type. Mulch macrofauna also showed higher abundance in the presence of lime in all cases, while mulch macrofauna richness and Shannon index were significantly increased by liming in converted fields (Fig. 15 and Table S8). The highest average macrofauna intensity (147 ± 16 individuals/m²) was observed in mulch materials collected from the converted-lime tea plots, followed by the abundance of mulch macrofauna from the non-converted lime plantations (118 ± 21 individuals/m²). The original non-limed soils, with an average of 63.9 ± 9 individuals/m², had the lowest macrofaunal abundance (Fig. 15). In mulch materials, 13 different macrofauna groups were found, while in soils were 12 groups, in which snails were not present (Table S9). Among these groups, earthworms were the most abundant, accounting for 30.7 and 20.5 individuals/m² in soil and in mulch on average,

respectively. The abundances of millipedes and centipedes, which were the second most abundant groups, were significantly greater in organic mulch, while the abundance of earthworms, spiders and insect larvae were significantly higher in the soils (Table S9). Soil macrofauna abundance was significantly different in response to different land use history ($P = 0.026$), but other biological indices were not statistically different, either with soil or mulch macrofauna.

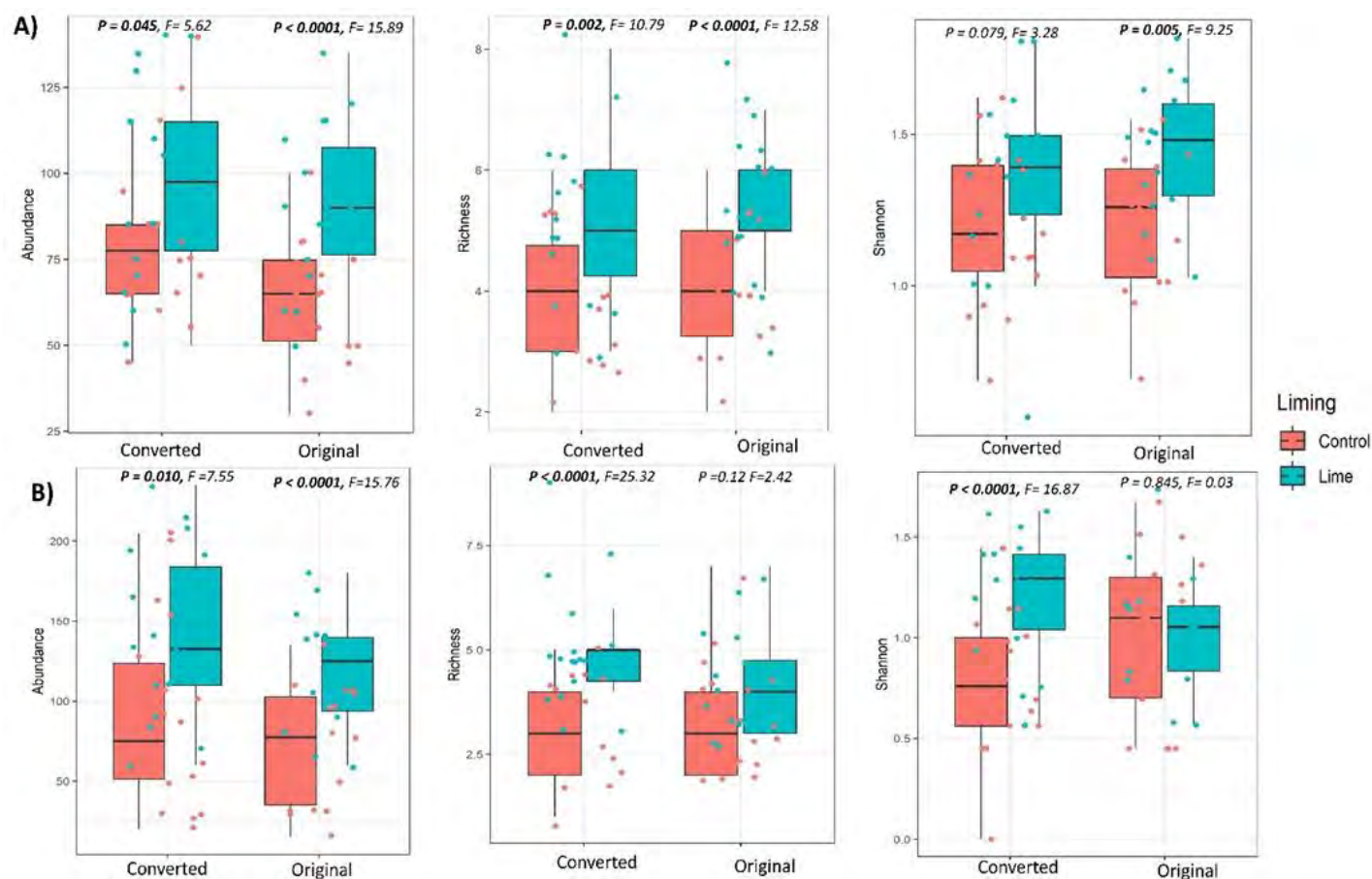


Figure 15. Changes in abundance and diversity of the soil macrofauna (A) and mulch macrofauna (B) as affected by liming treatments. Significance (P value) of liming effect on the land type treatments (converted and original) from the one way ANOVA test over the community abundance and diversity indices are presented

Additionally, the interaction of liming and land type had a significant effect on mulch macrofauna richness and Shannon index ($P < 0.05$, Table S2), but did not significantly affect soil macrofauna abundance, richness and Shannon index. Interestingly, neither liming nor land type had any significant impact on either soil nor mulch macrofauna community composition (P values were 0.065 and 0.12, respectively, PERMANOVA test, Fig. S1, supplementary data). Liming also significantly increased the abundance of springtails in soil, but reduced the intensity of termites observed in both soil and mulch (Table S9). By contrast, the figures of the remaining groups were not significantly different, regardless of lime application or land conversion. Based on soil factors and macrofauna groups, the PCA analysis showed that earthworms were closely correlated with soil pH and P availability, while millipede has a significant correlation with soil Al^{3+} ($P < 0.05$, Pearson test, Fig. 16 and Table S4).

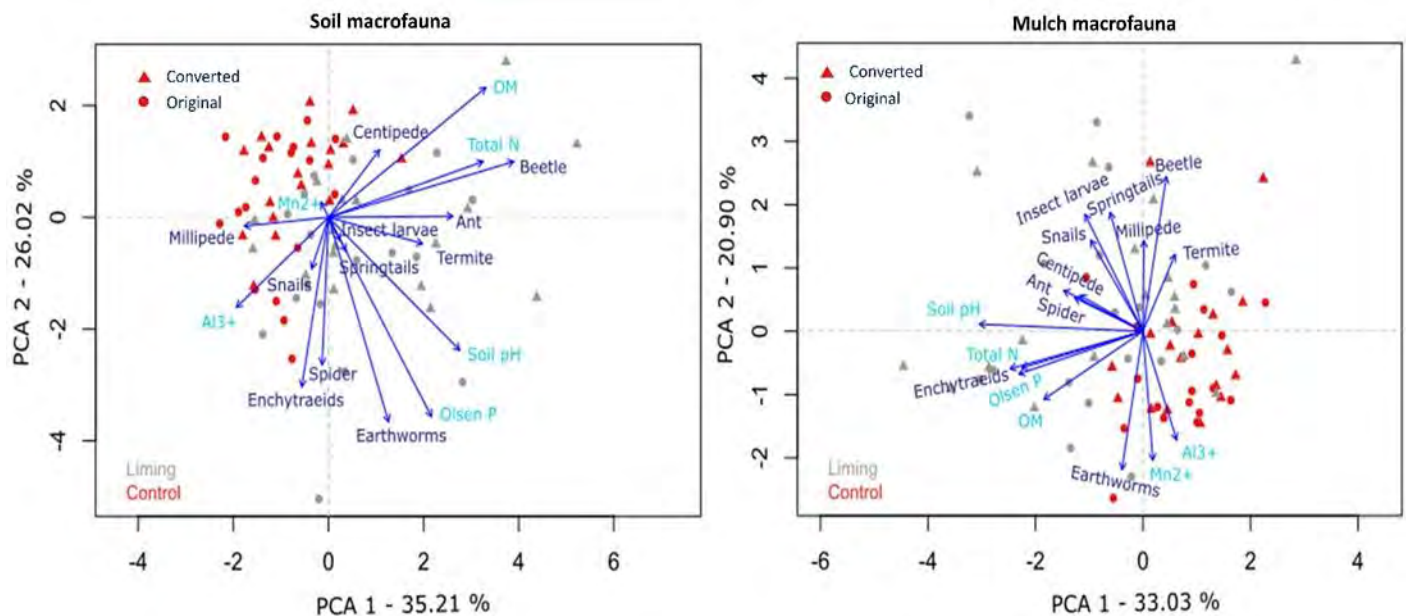


Figure 16 . Principal component analysis (PCA) indicates the correlations between soil and mulch macrofauna groups with soil characteristics collected from liming and land type treatments, each point represents a single sample.

For macrofauna collected from organic mulch, lime addition and land conversion both led to a significant difference in the abundance of millipede, centipede, and enchytraeids (Table S9). Millipede groups also have significant correlation with soil Olsen P and earthworms, while Enchytraeids were closely correlated to soil pH, Olsen P and the centipede group, as confirmed by the PCA analyses (Fig. 16 and Table S11).

3.4.3 Soil bacterial community composition, richness and diversity

After sequence data processing, a total of 17,341,432 quality filtered reads were retained for bacterial community datasets, with a minimum of 91,289 sequences per sample, resulting in 3,660 OTUs which were classified in 728 families and 41 phyla. At the phylum level, bacterial communities were dominated by the Proteobacteria ($40.4 \pm 4.6\%$) and Acidobacteria ($21.9 \pm 2.5\%$), followed by Thaumarchaeota ($6.5 \pm 0.48\%$) and Actinobacteria ($5.5 \pm 0.64\%$). The most abundant classes included Alphaproteobacteria ($14.6 \pm 1.61\%$), Gammaproteobacteria ($14.1 \pm 1.48\%$), Holophagae ($12.2 \pm 1.36\%$) and Betaproteobacteria ($11.2 \pm 1.25\%$) (Fig. 17). Non-metric multidimensional scaling (NMDS) and PERMANOVA analyses indicated that bacterial community composition was significantly different by rice paddy to-tea land conversion ($P < 0.001$, $R^2 = 0.44$, PERMANOVA test), and the interaction between soil conversion and lime addition ($P < 0.001$, $R^2 = 0.27$), but did not differ significantly due to solely lime application ($P = 0.174$, $R^2 = 0.013$, PERMANOVA test) (Fig. 18 and Table S12). Soil conversion implementation significantly reduced the total of soil bacterial OTUs by 6.7% (± 0.88 , ANOVA $P < 0.05$), irrespective of lime amendment (Fig. 18). Also, soil bacterial richness, Shannon and Simpson indexes were varied significantly by soil conversion (ANOVA $P < 0.05$), while the effect of liming on these indices was not significant (ANOVA $P > 0.05$) (Table S14). We also observed that soil bacterial abundance is positively linked to soil total N but significantly and negatively correlated to exchangeable Al^{3+} and Mn^{2+} (Fig. 24 and Table S15).

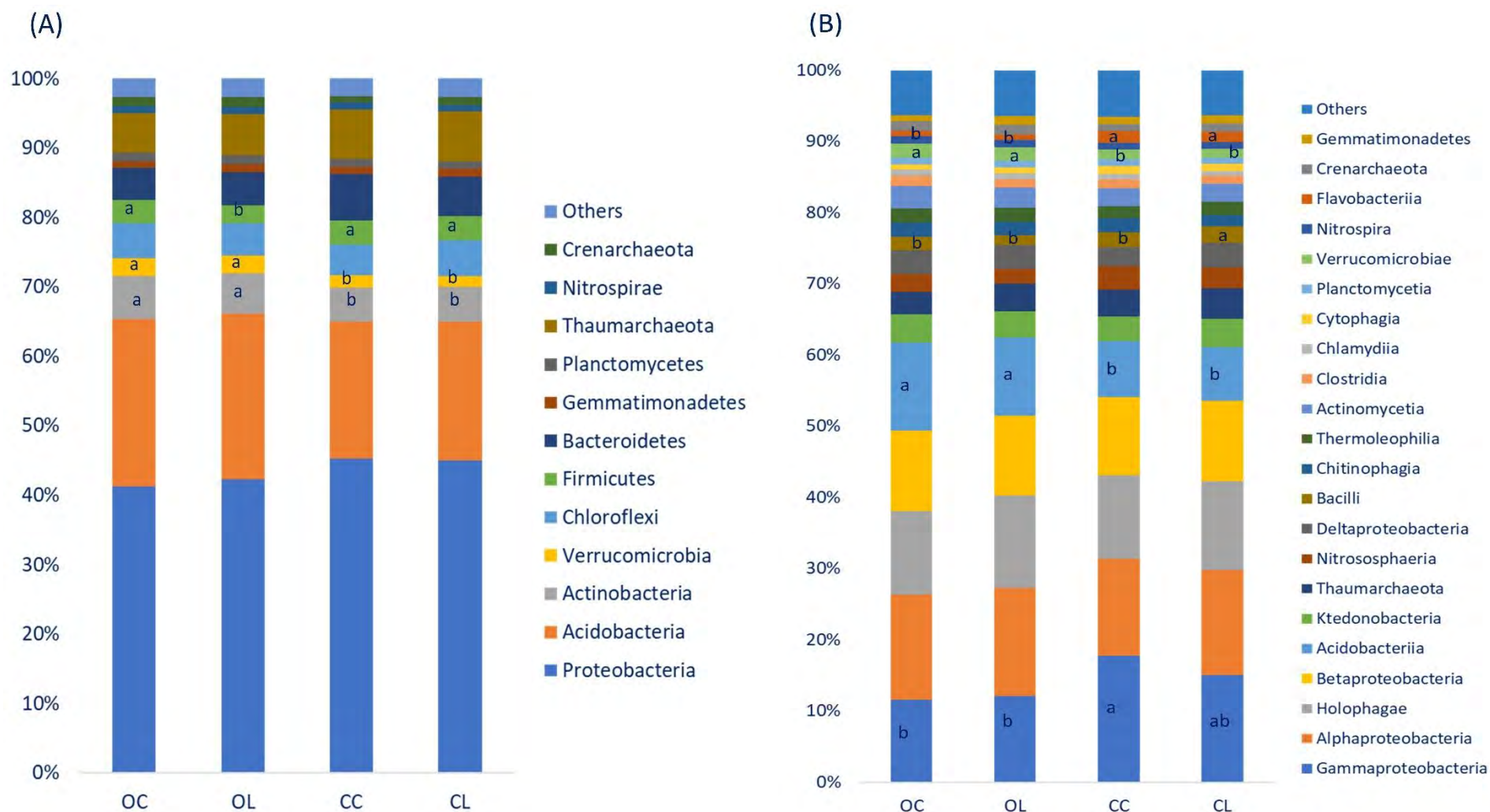


Figure 17. Composition of the soil bacteria at the phylum level (A) and class level (B) observed in the lime and land type treatments. In each treatment, soil fungal phylum and class means accompanied by different letters differ significantly at $P < 0.05$ (pairwise comparisons using the Tukey (HSD) test). OC = Original control, OL= Original lime, CC = Converted control, CL = Converted lime

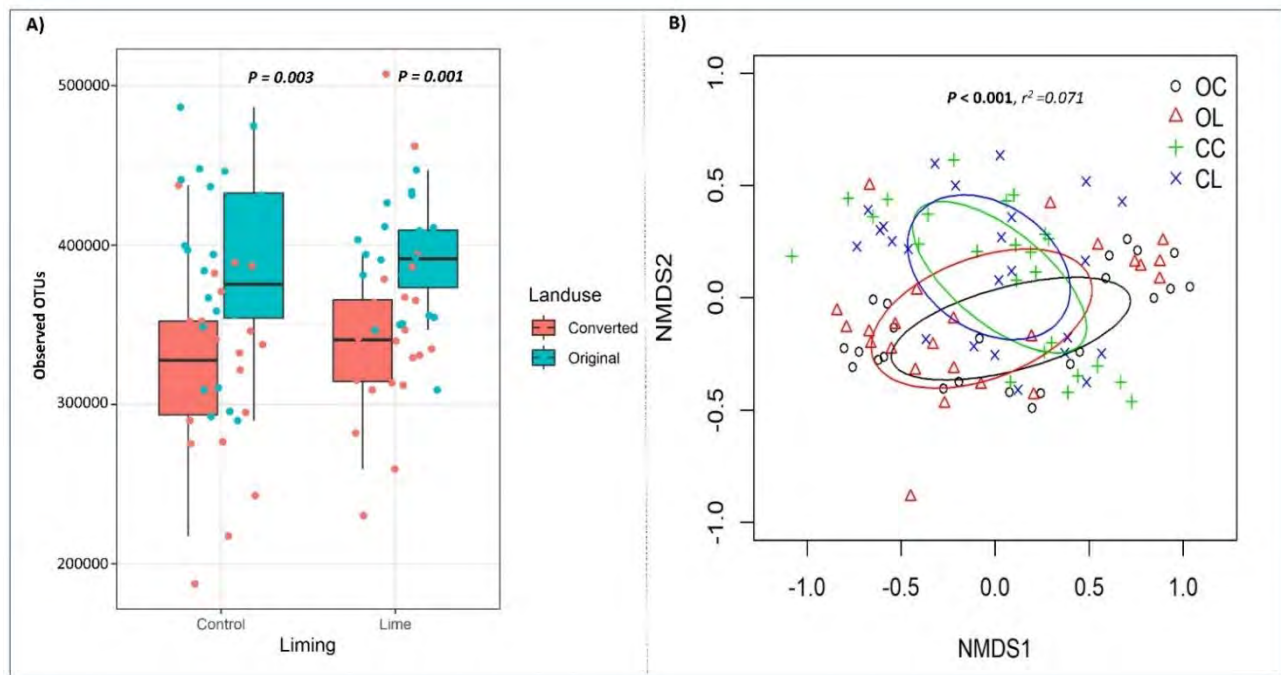


Figure 18. Box plot (A) and non-metric multidimensional scaling (NMDS) plots indicate the impact of liming and land type on soil bacterial communities. OC = Original control, OL= Original lime, CC = Converted control, CL = Converted lime. The significance (*P value*) of each grouping factor from the ANOVA (box plot) and PERMANOVA (NMDS) over the community dissimilarity matrices are shown

Likewise, apart from Shannon index, the combination of lime addition and soil conversion did not have any significant impact on soil bacterial evenness and richness (ANOVA, $P > 0.05$) (Table S6). Applying lime to original tea plantations resulted in the highest soil bacterial evenness (0.84 ± 0.09) and richness ($3,860 \pm 371$), as well as Shannon index (6.9 ± 0.66). In contrast, the lowest figures for the aforementioned indicators were observed in converted tea soils that did not apply lime.

For bacterial taxa, 46.3% of soil microbial phyla (19/41) were significantly influenced by land type, regardless of lime treatments, such as Asproteobacteria Acidobacteria, Actinobacteria, Verrucomicrobia and Firmicutes (ANOVA $P < 0.05$), while there were 35.5% (38/107) of soil microbial classes were significantly responsive to different land types. By contrast, only 2 bacterial phyla and 5 classes were significantly affected by lime application. Liming and land type

interactions have variable and quite limited effects on soil bacterial composition. Planting tea in soils that were converted from paddy fields significantly reduced the relative abundance of Actinobacteria, Verrucomicrobia, while increasing the relative abundance of Firmicutes (ANOVA $P < 0.05$). At class level, liming and land type interaction significantly increased relative abundance of Gammaproteobacteria but reduced the relative abundance of Acidobacteria, Clostridia and Chlamydiia.

3.4.4 Fungal community composition, richness and diversity

A total of 3,655,724 quality filtered reads were retained for fungal community datasets, resulting in 4,889 OTUs which were classified in 531 families and 39 phyla. At the phyla level, Ascomycota and Basidiomycota were the dominant groups, representing 42.8% (± 4.67) and 20.4% (± 2.16), respectively, followed by Mucoromycota (15.5 $\pm 1.52\%$) and Platyhelminthes (4.85 $\pm 0.44\%$). The most abundant fungal classes were Sordariomycetes and Agaricomycetes, which accounted for 17.5% (± 1.72) and 17.2% (± 1.66), followed by Mortierellomycetes (14.5 $\pm 1.39\%$) and Eurotiomycetes (7.5 $\pm 0.89\%$) (Fig. 19). Soil fungal community richness and composition were significantly varied by the conversion from paddy farms to tea plantations, and by the interaction of liming and soil conversion, as shown by the box plot, NMDS and PERMANOVA analyses ($P < 0.001$, $R^2 = 0.101$), but was unaffected by lime application (PERMANOVA $P = 0.273$, $R^2 = 0.01$) (Fig. 19 and Table S12). In contrast to soil bacterial composition, soil fungal total OTUs observed in newly established tea plantations was significantly increased by 16.8% (± 1.65 , ANOVA $P < 0.001$). Fungal community evenness, richness, Shannon and Simpson indexes were all significantly influenced by land type (ANOVA $P < 0.05$), but were not significantly affected by either lime application or the interaction effect of liming and soil conversion implementation (ANOVA $P > 0.05$) (Table S13).

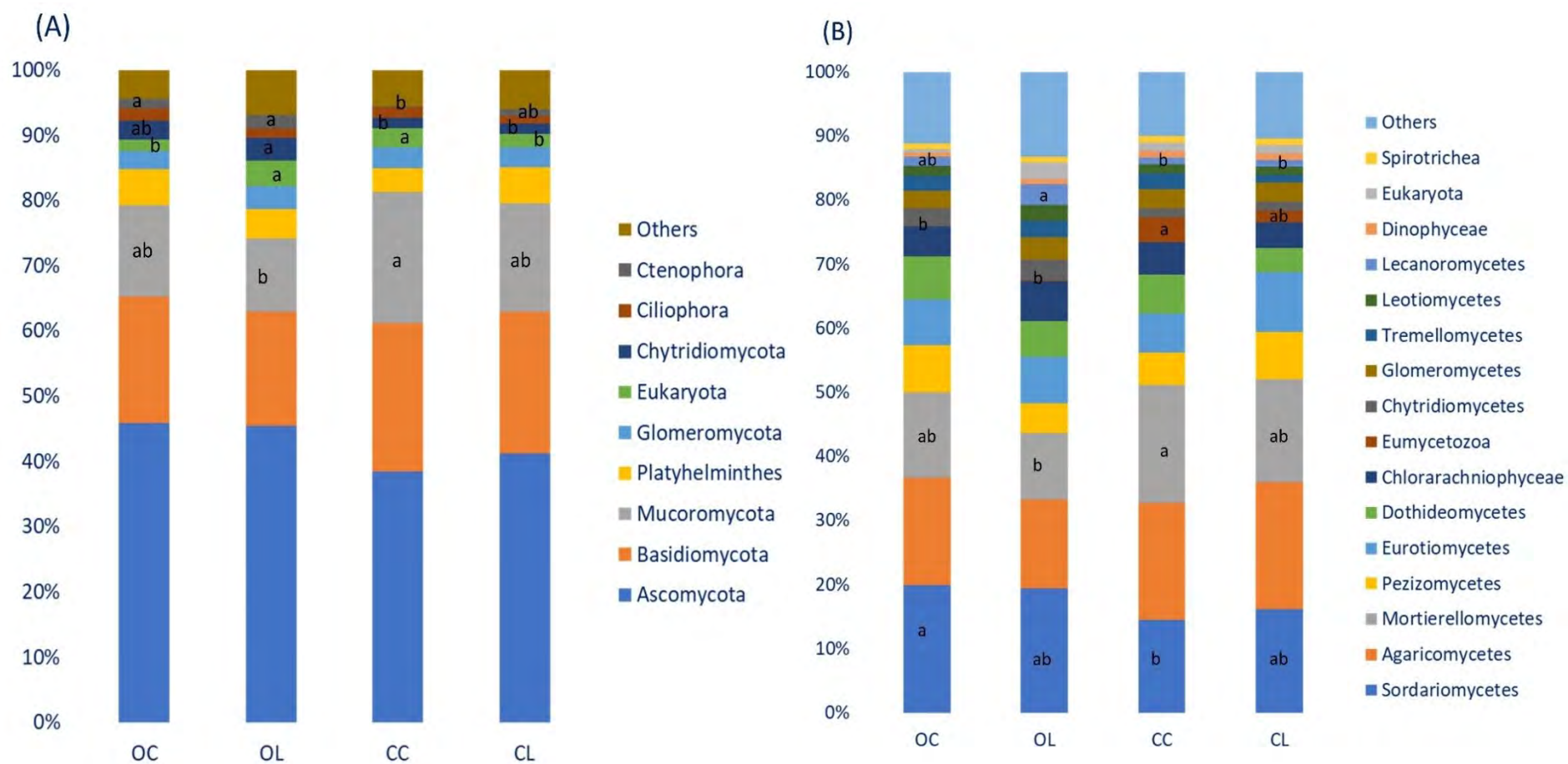


Figure 19. Soil fungal composition at the phylum level (A) and class level (B) observed in the lime and land type treatments. In each treatment, soil fungal phylum and class means accompanied by different letters differ significantly at $P < 0.05$ (pairwise comparisons using the Tukey (HSD) test). OC = Original control, OL= Original lime, CC = Converted control, CL = Converted lime

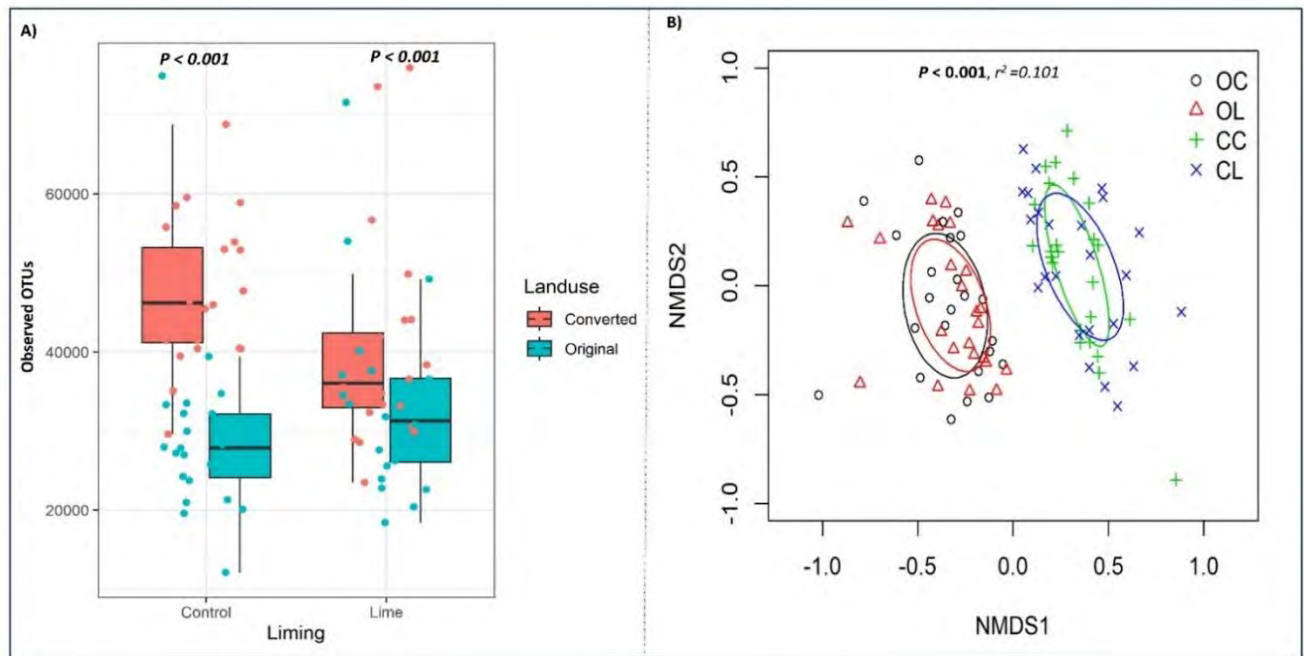


Figure 20. Box plot (A) and non-metric multidimensional scaling (NMDS) plots indicate the impact of liming and land type on soil fungal communities. OC = Original control, OL= Original lime, CC = Converted control, CL = Converted lime. The significance (*P value*) of each grouping factor from the ANOVA (box plot) and PERMANOVA (NMDS) over the community dissimilarity matrices are shown

Planting tea in the soils that converted from paddy fields also resulted in greater impacts on soil fungal taxa than lime application. Of the 39 phyla, 9 fungal phyla ($23 \pm 2.45\%$) were significantly affected by different land type, such as Sordariomycetes, Mortierellomycetes, Chlorarachniophyceae, Eumycetozoa and Lecanoromycetes, while there were only 2 phyla (Mucoromycota and Ctenophora) significantly responsive to lime incorporation. For soil fungal community classes, the relative abundance of 16/101 classes were significantly different as the consequences of soil conversion from paddies to tea plantations, while almost none was influenced by lime application.

3.4.5 Tea root and soil AMF communities

Tea root AMF colonisation frequency and intensity

Lime amendment significantly increased tea root AMF frequency and intensity ($P < 0.05$, ANOVA test) (Fig. 21). Root AMF frequency increased by around 4% because of lime addition, both in original and converted tea plantations. Similarly, liming increased tea root AMF intensity by 4 -7% on average, and the increase was greater in converted tea soils than in non-converted tea plots. In addition, soil conversion significantly affects tea root AMF intensity, but AMF frequency was unaffected (Fig. 21 and Table S14).

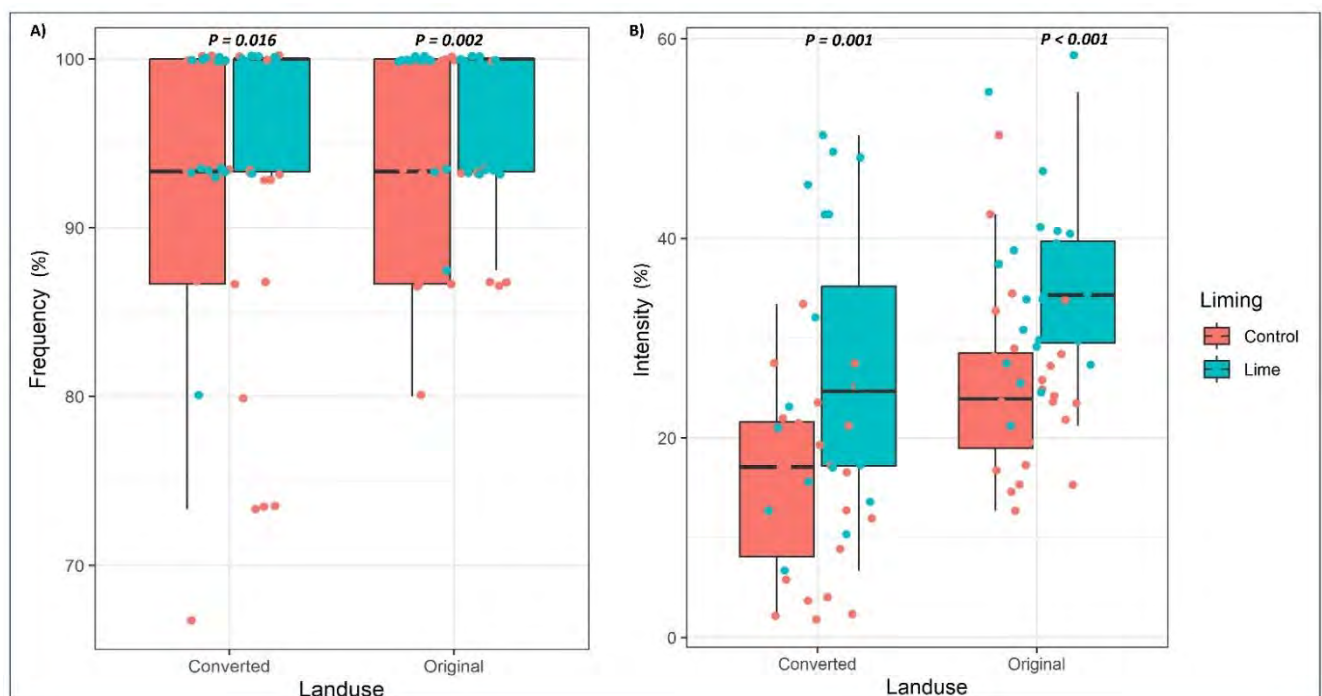


Figure 21. Response of tea root AMF frequency (A) and intensity (B) to liming and soil conversion practices. Significance of each grouping factor from one-way ANOVA test is indicated

Soil AMF community composition, richness and diversity

Nearly 4,652,000 quality filtered reads were retained for the soil AMF community dataset, which

was classified into 60 AMF virtual taxa (VT), 18 phyla and 41 classes (Fig. 22). The most dominant AMF phylum found in tea soils was Glomeromycota ($48.4 \pm 4.92\%$), followed by Chlorophyta ($28.8 \pm 3.22\%$) and Mucoromycota ($11.5 \pm 1.61\%$). Likewise, Glomeromycetes, Ulvophyceae and Mortierellomycetes were the dominant tea soil AMF classes, accounting for $50.8\% (\pm 0.56)$, $21.5\% (\pm 0.27)$ and $10.0\% (\pm 0.12)$, respectively. Land type also had a greater impact on soil AMF taxa than lime incorporation. Of the 18 soil AMF phyla, 5 phyla such as Chlorophyta, Zoopagomycota and Mucoromycota were significantly responsive to soil conversion from paddy to tea cultivation, while that of AMF classes were 15/41, including Ulvophyceae, Zoopagomycetes, Tremellomycetes, Chlorophyceae and Saccharomycetes. In contrast, only 1 soil AMF phyla (Mucoromycota) and 2 classes (Mortierellomycetes and Leotiomyces) were significantly affected by lime addition (Fig. 22).

Land type and its interaction with liming significantly influenced soil AMF compositional community, indicated by the NMDS assessment and PERMANOVA test ($P < 0.001$, Fig. 23 and Table S12). Land type treatment also significantly affected soil AMF composition, but interestingly, the effect on the total AMF OTUs was only significant with the un-limed soils. On the other hand, the lime amendment did not induce any significant changes in the soil AMF richness and composition. Soil AMF biological indicators including evenness, richness, Shannon and Simpson index were all significantly altered after soil conversion from paddy fields to tea farms, but these indices were not strongly responsive to either lime amendment or the interaction of liming and land conversion (Table S14). In addition, all of these biological indices were greater in non-converted compared to converted tea plantation soils. The highest values of soil AMF evenness and richness were observed in original un-limed soils, accounting for $0.65 (\pm 0.072)$ and $389 (\pm 41.8)$ respectively. Similarly, soil AMF Shannon and Simpson index ranged from 3.4 and 0.89 (Converted control), respectively, to 3.88 (Original control) and 0.95 (Original lime), respectively.

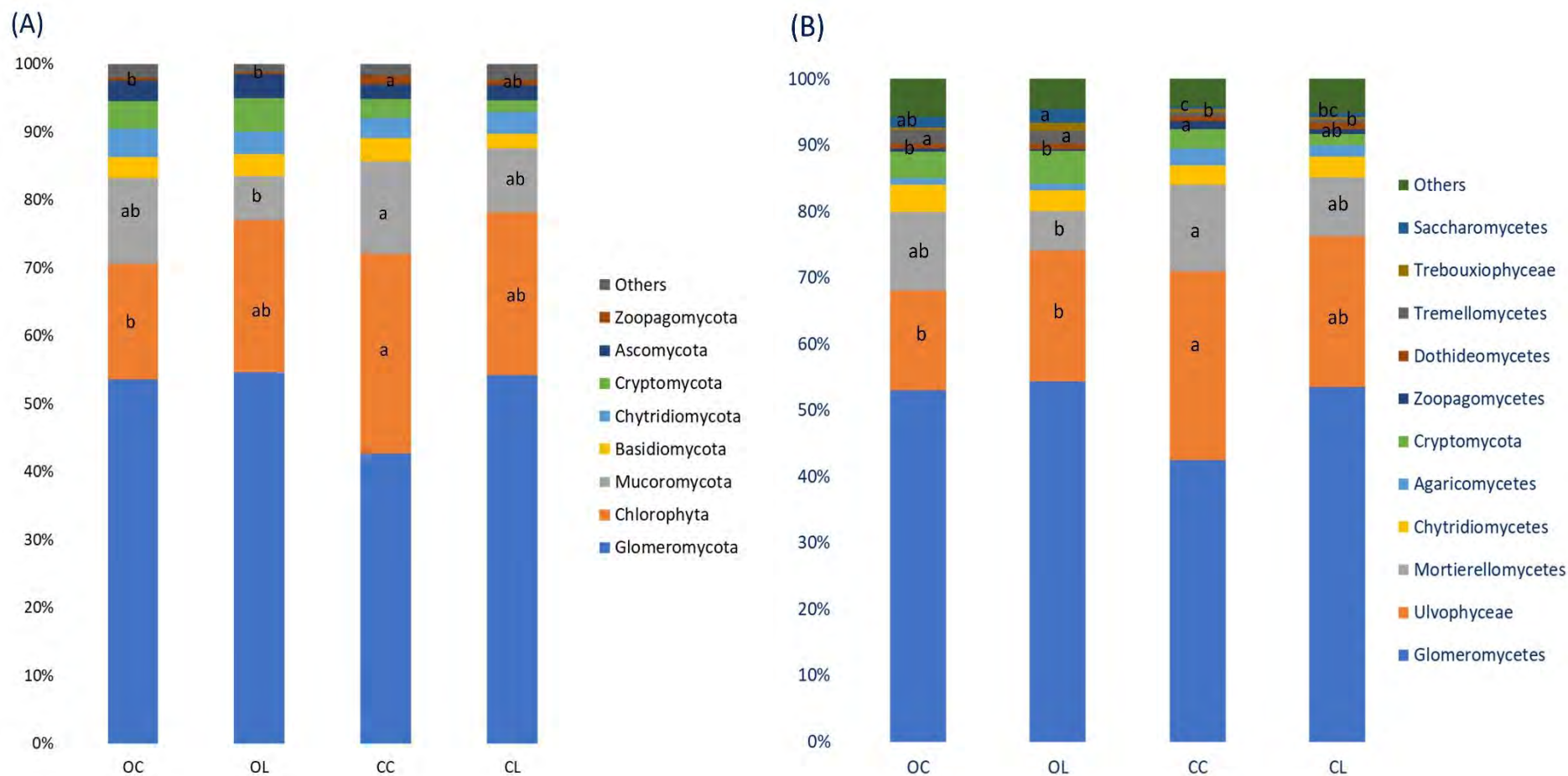


Figure 22. Soil AMF composition at the phylum level (A) and class level (B) observed in the lime and land type treatments. In each treatment, soil AMF phylum and class means accompanied by different letters differ significantly at $P < 0.05$ (pairwise comparisons using the Tukey (HSD) test). OC = Original control, OL= Original lime, CC = Converted control, CL = Converted lime

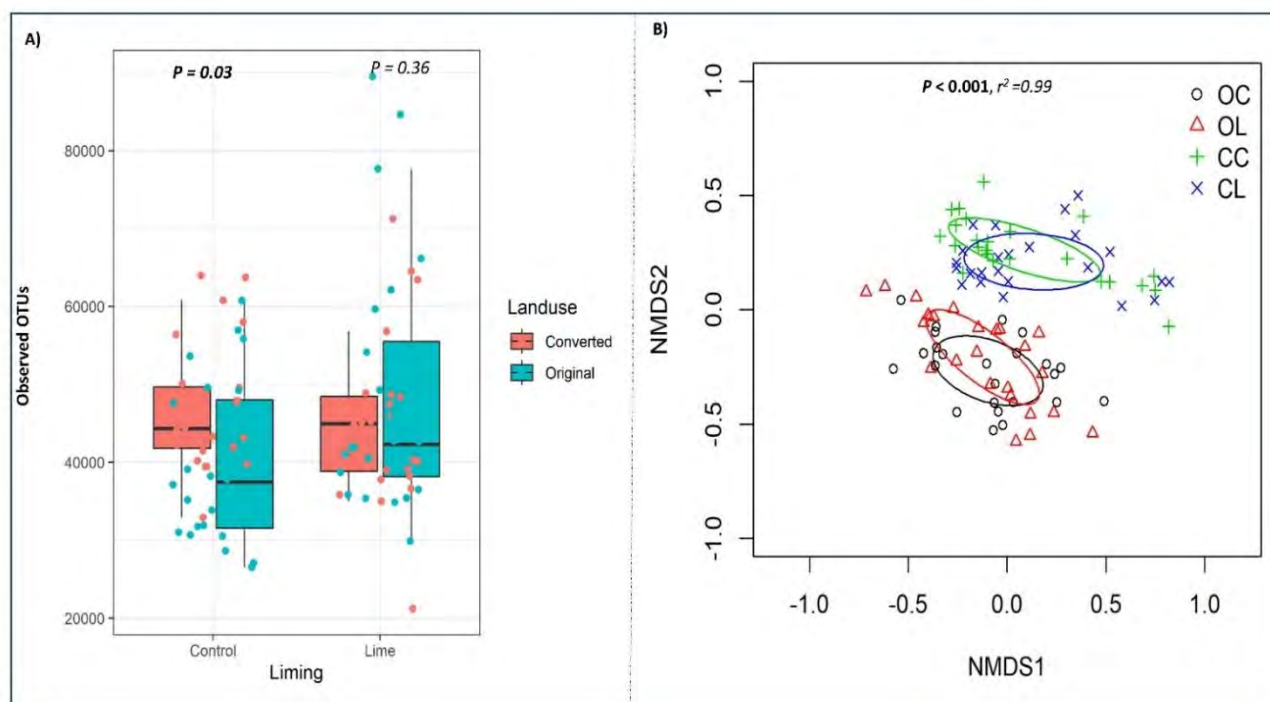


Figure 23. Box plot (A) and non-metric multidimensional scaling (NMDS) plots indicate the impact of liming and soil conversion practice on soil AMF communities. OC = Original control, OL= Original lime, CC = Converted control, CL = Converted lime. The significance (P value) of each grouping factor from the ANOVA (box plot) and PERMANOVA (NMDS) over the community dissimilarity matrices are shown

3.4.6 Tea yield and yield components

Tea yield and yield components observed in limed soils from 2021-2022 were consistently greater than in non-limed control treatments, even though the effect was not always significant (Table 9).

In 2021, tea shoot density observed in lime plantations was significantly higher than in non-limed treatments, but the means of shoot weight and tea yield were not statistically different between lime and non-limed plantations. In the following year, lime application significantly enhanced tea yield and yield components, regardless of soil conversion. The lowest yield was observed in original non-limed tea plantations ($14.34 (\pm 1.62)$ tons ha^{-1} year $^{-1}$), while the highest tea yield was recorded

in tea soils that practiced both conversion and liming (15.61 (± 1.75) tons ha⁻¹ year⁻¹). Limed tea plots also had the highest tea shoot density and shoot weight, accounting for 660 shoots/ m² and 35.36 g in the original lime and converted lime plots in 2022, respectively (Table 9). By contrast, non-limed tea plantations produced the lowest tea shoot density and an average weight of 100 tea shoots, which were 587 shoots/ m² (original plots) and 33.00 g (converted tea farms) in 2022.

Table 9. Tea yield and yield components as affected after 9-month lime application (lime vs control) and different land types (converted and non-converted) (mean \pm SD). Different letters indicate significant changes among treatments, according to the Tukey (HSD) tests

Treatments	2021			2022		
	Shoot density (shoots/ m ²)	Shoot weight (100 shoots)	Yield (tons/ha)	Shoot density (shoots / m ²)	Shoot weight (100 shoots)	Yield (tons/ha)
Original control	587 \pm 65b	33.41 \pm 3.38a	14.04 \pm 1.16a	609 \pm 65b	33.86 \pm 3.12ab	14.34 \pm 1.62b
Original lime	623 \pm 79b	34.05 \pm 3.52a	14.60 \pm 1.12a	660 \pm 85a	35.09 \pm 4.21ab	15.27 \pm 1.66a
Converted control	611 \pm 68ab	33.00 \pm 3.41a	14.42 \pm 1.11a	622 \pm 74b	33.48 \pm 3.91b	14.73 \pm 1.51 ab
Converted lime	628 \pm 71a	33.60 \pm 3.65a	15.08 \pm 1.29a	648 \pm 79a	35.36 \pm 4.09a	15.61 \pm 1.75a

The correlations between tea yield and soil chemical and biological indicators are assessed by a joint PCA, as presented in Fig. 24. The first two axes together explained nearly 64% of the cumulative variability. Tea yield is significantly and positively correlated to soil chemical variables including soil pH, total N, and Olsen P, as well as macrofauna, bacterial and tea root AMF colonization, but negatively linked to soil AMF abundance ($P \leq 0.05$, Person test, Fig. 24 and Table S15).

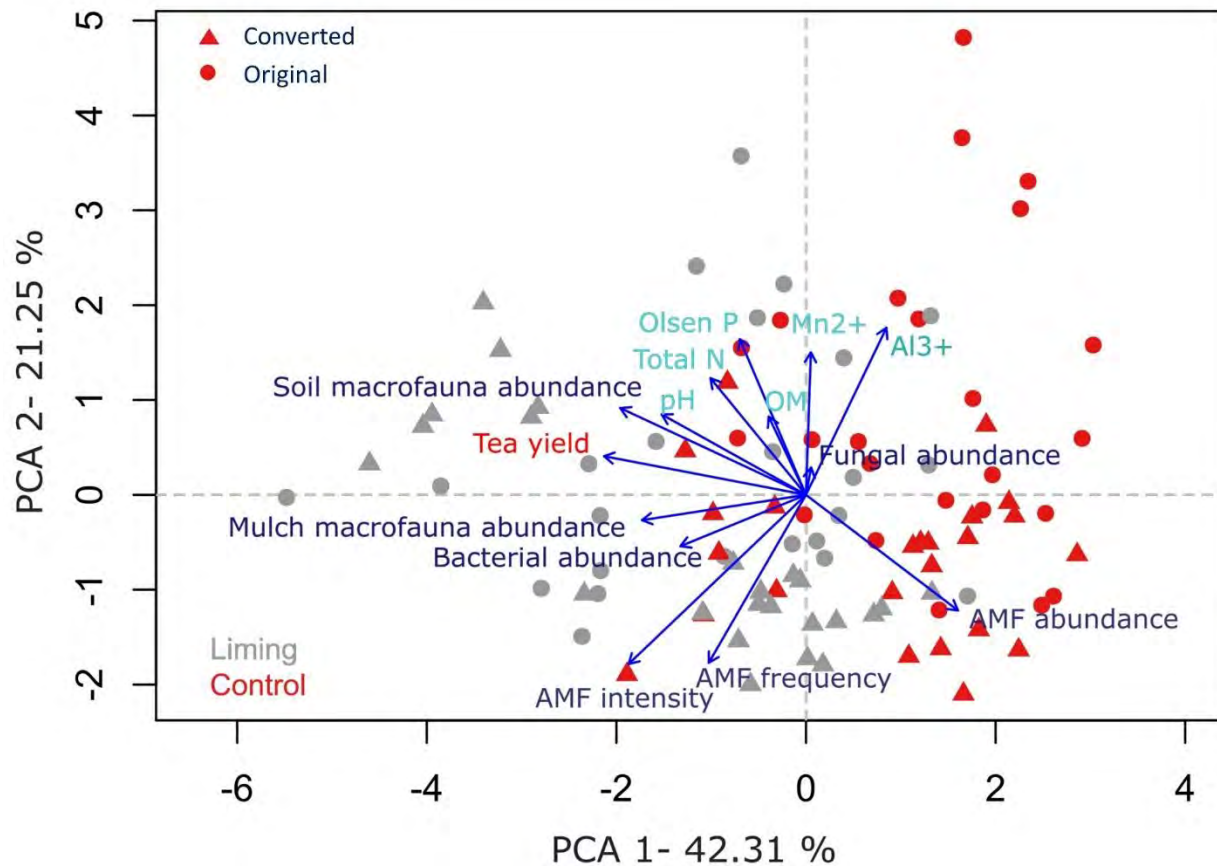


Figure 24. Principal component analysis (PCA) indicates the correlations between tea yield and soil characteristics, tea root colonization as affected by lime application and land type treatments in agroecological tea plantations, each point represents a single sample

3.5 Discussion

3.5.1 Lime-induced effects on macrofauna

Generally, invertebrates have a limited ability to adapt to soil acidity, and they tend to concentrate in sites where soil pH is more favorable such as in leaf litter or in the rhizosphere rather than in the “bulk” soil (Lavelle et al. 1995). This could be the case in our study since we found that all studied tea plantation soils were strongly acidic as a result of long-term tea cultivation, and with low levels of macrofauna diversity compared with previous studies (Lavelle et al. 2022; Yu et al. 2021). Nine

months after the application, liming significantly enhanced both soil and mulch macrofauna biological indices, including abundance, richness and Shannon index, which could be mainly driven by an increase in soil pH and OM content, as a result of a lime-induced effect. Soil pH has been identified as the key driver of soil animals, and appropriate pH levels can positively affect the decomposition of organic matter and the cycling of nutrients in soils, thus benefiting soil organisms (Kuśmierz et al. 2023; Ochoa-Hueso et al. 2014). This hypothesis is confirmed by the PCA analysis indicating that both soil and mulch macrofauna abundance is significantly and positively correlated with soil pH analyzed in this study (Fig. 24 and Table S15). By contrast, strongly acidic soil (e.g. pH < 4.0) often has an adverse effect on macrofauna abundance (Johnston and Sibly 2018; Korboulewsky et al. 2016). Our findings are consistent with previous studies which reported that liming has positive effects on the abundance and diversity of earthworm and enchytraeid communities (Cole et al. 2006; Lavelle et al. 1995; McCallum et al. 2016).

In our study, lime incorporation had a significant positive effect on abundance of earthworms and centipedes, while negatively correlating to termite groups. Previous investigations also showed the positive effects of liming on earthworm survival rate, mobility, density, and productive outputs (Cole et al. 2006; Moore et al. 2013). By contrast, termites are known to have the ability to survive in low pH environments, and liming has been considered as an effective strategy to reduce termite activity and reproduction (Kagezi et al. 2010; Sileshi et al. 2009). We also observed that in acidic tea plantation soils, earthworms, millipedes and centipedes are dominant faunal groups. This findings are in line with recent studies (Jamatia and Chaudhuri 2017; Wang et al. 2018b), which revealed that earthworms, millipede and centipedes are among the most abundant invertebrate groups in acidic soils, which play important roles in the soil physicochemical and biological processes, and are essential indicators of soil health status. For instance, earthworms and millipede generally serve as soil conditioners and can enhance soil nutrient cycling through the rapid

incorporation of organic matter into mineral soils, which subsequently promote soil microbial growth and plant productivity (Ahmed and Al-Mutairi 2022; Bhadauria and Saxena 2010; Snyder and Hendrix 2008).

3.5.2 Soil chemical properties in association with liming and land conversion

After nine months since the application, we found that lime amendment significantly increased soil pH in the acidic tea soils by 0.4 units on average. Lime (CaCO_3) and other liming sources such as dolomite reduce soil acidity due to their neutralization effectiveness in acid soils, in which the carbonate components react with hydro-ion in soils and subsequently increase the soil pH (Du Toit et al. 2022; Mahmud and Chong 2022). Positive effect of liming on soil pH has been observed in tea plantations (Hirono and Nonaka 2014; Xue et al. 2010) as well as other cropping systems (Holland et al. 2018; Li et al. 2019b). Smith and Hardie (2022) also showed that liming amendment with the application rate of 1 and 2 tons/ ha significantly shifted tea soil pH by 0.9 units (from 4.2 to 5.1) and 1.7 units (from 4.2 to 5.9), respectively, and the effect of lime incorporation in increasing soil pH was highest during the first 2 years and decreased thereafter. In this study, soil exchangeable Al^{3+} and Mn^{2+} were negatively correlated with lime application, indicating that liming could be an effective way to reduce soil Al and Mn toxicity, which has been a serious concern in acidic tea plantation soils (Ni et al. 2018; Yan et al. 2018). This, agreed with previous studies which shown that liming adds calcium and magnesium to the soil thus raising the soil pH and forcing the soluble aluminum and manganese to convert into non-toxic (solid) chemical forms (Dinkecha and Tsegaye 2017; Merlos et al. 2023). We also observed soil Olsen P in limed plots increased by around 16% on average, suggesting a clear effect of liming on soil P availability. This is plausible since a significant increase in soil pH following liming might have increased in the P

bioavailability. Our synthesis is supported by a significant correlation between soil pH and Olsen P availability (Table S4), and consistent with results from recent studies by Olego et al. (2022) and Simonsson et al. (2018). In addition, as soil pH increasing as consequences of liming in acidic soil environment, Al and Fe oxides could become more negatively charged, thereby contributing to the phosphate ion desorption from mineral surfaces and increases in the soil P availability (Mkhonza et al. 2020). It has been reported that liming effect in reducing soil acidity was significantly greater in the surface soils, and over time the effect may influence soil pH in deeper soil layers, which is mainly driven by precipitation and other soil chemical processes (Lewis et al. 2018; Yin et al. 2021a). Further work considering the sampling at deeper soil layers could therefore provide a more comprehensive picture of how liming contributes to control soil acidification.

With regards to the newly established tea plantations, soil pH values were significantly higher compared to the well-established (non-converted) tea soils. This finding is corroborated by several studies (Hui et al. 2010; Li et al. 2016) who concluded that increasing tea plant age resulted in an increase of organic and carbonic acids induced by tea roots into the rhizosphere, which facilitate soil acidification. In tea plantations, soil pH in the topsoil naturally decreased by 0.071 units per annum, and the values following 13, 34 and 54 years of tea cultivation were 1.1; 1.62 and 2.07 units respectively (Hui et al. 2010; Ni et al. 2018). In our study, the original soils have been used for tea cultivation for more than 30 years, which contributed to a greater level of chemical compound accumulation in tea soils. Furthermore, intensive uses of nitrogen fertilizers of tea farmers in the studied region to ensure a satisfying tea yield and soil nutrient loss replacement in the previous tea generations could be another reason contributing to the significant difference in the soil pH of converted and non-converted tea soils (Viet San et al. 2021). We also observed that the levels of Al^{3+} in converted tea soils were significantly greater than that in original soils, even though converted tea soils were less acidic (soil pH values were significantly higher). This is

possibly due to the existing amount of soil Aluminum in paddies was far greater than in original soils when they were converted, leading to a significantly different level between converted and original tea soils. Our hypothesis is supported by Zhao et al. (2017) who reported that both free-form and amorphous Aluminum oxides in paddy soils (1.54 ± 0.25 and 1.43 ± 0.25 g kg⁻¹, respectively) were higher compared to upland systems (1.41 ± 0.33 and 1.10 ± 0.20 , respectively).

3.5.3 Soil microbial communities associated with liming and land use history

Soil microbes play essential roles in the soil biogeochemical cycles, and are generally sensitive to changes in both biotic and abiotic factors in the environments inhabit (Wei et al. 2020; Yan et al. 2021b; Zhang et al. 2021). Among soil management strategies, land use history and liming could have strong impacts (both positive and negative) on soil microbial compositional and structure communities, and it has been concluded that land use change and locations have a greater effect on soil microbial community structure compared to lime application (Schroeder et al. 2018; Xue et al. 2010; Yin et al. 2021b). These findings support our preliminary results obtained nine months after lime incorporation which indicated that apart from root AMF colonization, soil bacterial, fungal, and AMF communities are mostly driven by land use history, and they are less responsive to liming application.

3.5.4 Land use history and soil microbial community diversity and composition

In the present study, tea soils that were converted from paddy fields significantly reduced the bacterial OTU richness, while increasing that of soil fungal and AMF communities. Soil conversion also significantly affected the compositional community of soil bacteria, fungi and AMF observed in tea plantation soils. These findings are in agreement with previous studies (Wu et

al. 2020; Yang and Zhang 2014) who illustrated that converting from paddy fields to orchard farms significantly influenced soil microbial community composition and structure, based on the combined phenotypic analyses. Likewise, Yuan et al. (2015) revealed that soil fungal PLFAs were significantly increased by more than ten times, following the conversion from rice paddies to vegetable farms. Different types of agricultural land use have different management practices, which could significantly correlate to soil chemical properties and variation in microbial communities (Lee et al. 2020; Rampelotto et al. 2013; Tian et al. 2017). Converted tea soils in our study were used to grow paddy rice for more than 20 years, with high intensity of transplantation, tillage, cultivation and different fertilization regimes. Compared with original soils, tea soils that converted from paddy fields were also submerged for a long time. These different management practices result in changes of soil physicochemical conditions and biological processing, affecting the living environment for soil microorganisms (Suleiman et al. 2013; Yang and Zhang 2014; Zhu et al. 2021). Specifically, soil chemical properties including pH, OM, available P and exchangeable Al^{3+} observed in converted tea soils from this work were significantly higher than in non-converted plots. Soil pH has been widely considered as a dominant factor in shaping the belowground community composition at a wide range of environmental conditions (Naz et al. 2022; Suleiman et al. 2013). The distribution of Acidobacteria, the dominant bacterial phylum found in this study for instance, is highly dependent on soil pH (Liu et al. 2016; Sheng et al. 2013). Higher inputs of P are also significantly associated with some copiotrophic taxa such as Proteobacteria (Schroeder et al. 2018; Yin et al. 2021a). Ascomycota is also the main fungal decomposer, and they play the biggest role in recycling plant residues (Wang et al. 2016; Ye et al. 2020; Zhang et al. 2020), so an increase in the soil OM due to land conversion could enrich this fungal and AMF genera. A negative significant correlation between soil Al^{3+} , Mn^{2+} and bacterial

abundance ($P < 0.05$, Pearson test, Table S15) further suggests that higher levels of these elements in converted soils could contribute to a reduction in the soil bacterial community composition.

3.5.5 Response of soil microbial communities to lime application

Liming effects on soil microbial community structure in general, soil bacterial diversity and functional community have been widely investigated, however, the research results were inconsistent, and the mechanisms regarding how liming affects soil microbial community remain unclear (Holland et al. 2018; Wang et al. 2021). Interestingly, we found that lime incorporation did not significantly affect soil bacterial relative abundance and compositional community, but strongly altered that of soil AMF. This finding is in general agreement with previous research which reported that liming had marginal or neutral effects on soil bacterial communities, and only a few bacterial families such as Cytophagaceae, Flavobacteriaceae and Intrasporangiaceae were correlated with lime incorporation (Tavi et al. 2013; Yin et al. 2021a). Similarly, Wang et al. (2013) revealed that lime addition (0.4 and 0.8g CaCO₃ per kg⁻¹ soil, pot experiment) did not induce any significant changes in the dominant bacterial community composition and abundance in acidic soil, despite a 0.3 unit increase in soil pH, which is similar to our investigation. Though soil pH has been widely considered a crucial predictor of soil bacterial community composition and diversity, and an essential factor affecting many soil chemical and biological processes (Lauber et al. 2009; Naz et al. 2022; Sheng and Zhu 2018), a small shift in soil pH following lime application could not be enough to induce a significant effect on soil microbial community observed within locations (Schroeder (Schroeder et al. 2018). Previous works also demonstrated that liming had neutral to limited effects on soil fungal relative abundance and composition (Al-Sadi and Kazerooni 2018; Narendrula-Kotha and Nkongolo 2017). Examining the impacts of liming on soil

fungal communities using 4 different liming rates (0, 673, 1345, and 2690 kg ha⁻¹), Yin et al. (2021b) noted that lime application did not significantly affect soil fungal diversity and richness, while fungal community composition was significantly affected by location and soil depth. However, this is not always the case of soil fungal diversity. For example, Xue et al. (2010) showed that the structural diversity soil microbial structure diversity index increased with the liming rate in all the experimental ecosystems, including tea orchards, the wasteland and the forest. Lin et al. (2018) also demonstrated that an increase in soil pH following lime or pig manure amendment altered fungal community diversity in Ultisols. From our field trials, liming increased soil pH by around 0.4 units, in the surface soil (0-20cm) and this increase may benefit some fungal taxa which are favored by relatively higher pH conditions (Rousk et al. 2010). In contrast, other fungi which are more adaptable to acidic soil may be suppressed (Wang et al. 2021; Yin et al. 2021b). Consequently, fungal diversity appeared to be unaffected by liming. We observed that liming increased the richness of some dominant fungal classes such as Sordariomycetes, Chlorarachniophyceae and Trematoda, while reducing the relative abundance of Agaricomycetes, Dothideomycetes and Mortierellomycetes. This hypothesis is in agreement with study by Kjølner and Clemmensen (2009) who revealed that the fungal species belonging to the genus *Tylospora* and the order Pezizales were significantly more frequent in limed soils, while species of the genera *Russula* and *Lactarius* decreased in frequency. Contrary to the soil microbial communities, liming was associated with a significant increase in the tea root AMF intensity and frequency, as recorded from our experimental study. This is consistent with observations made by Guo et al. (2012), Vázquez et al. (2020) and Heyburn et al. (2017). An enhancement in root AMF colonization following lime addition possibly due to the better growth of tea roots in the limed plots, which is of particular significance as AMF play a critical role in the uptake of plant-limiting nutrients (Johnson et al. 2005).

3.5.6 Tea yield and yield components response to liming

Liming can enhance crop productivity by primary effects on improving soil physicochemical and biological characteristics, which subsequently lead to increased availability and mobility of numerous essential nutrients for plant uptake (Agyin-Birikorang et al. 2022; Jaskulska et al. 2014; Li et al. 2019a). Under strongly acidic soil conditions ($\text{pH} < 4.5$), the availability of all mineral nutrients (Mn excluded) are reduced, and lime amendment, by raising the soil pH, will increase their availability to plants (Holland et al. 2018; White and Greenwood 2013). Liming also can reduce Al and Mn toxicity levels, one of the main factors causing soil nutrient imbalance and leaching, and deficiency in water and nutrient uptake of the root systems by inhibiting the expansion, elongation, and division of root cells (Venkatesan et al. 2010; Wang et al. 2015). Our study indicated that liming consistently enhanced tea yield and yield components, but the effect was only significantly different in the second year following lime application. Similar observations were also reported by Han et al. (2007) who illustrated that lime application increased tea yield by around 3%, and the effect of liming on the first year following CaCO_3 addition was not statistically different, but became significant during the second and third years. It is also documented that the major impact of superficial liming can be observed over a longer period, when the high reactivity of lime anions and the low solubility of lime with the acids in the soil layer where it is incorporated contribute to significant changes in soil chemical properties (Almeida et al. 2015). These findings suggest that longer term study might be needed to have a better understanding of liming efficiency on tea productivity. An appropriate liming rate could increase tea yield, number of tea buds per m^2 , and weight of 100 tea buds by 45.2%, 23.7%, and 17.0% respectively, while over-liming could restrict tea growth and productivity mainly due to the high soil pH and Ca concentration inhibiting the plant uptake of K and Al (Yan et al. 2021a). Overall, the positive changes of soil health

indicators as a result of lime application, as indicated by our joint analysis (Fig. 24 and table S15) would all contribute to an enhancement of tea productivity observed in this study.

3.6 Conclusions

Overall, we demonstrated that liming can be an effective strategy to reduce tea soil acidity and heavy metal toxicity risks, while enhancing soil OM and diversity of soil and mulch macrofauna communities. Further, lime incorporation also significantly enhanced tea root AMF colonization, but the impacts on soil bacterial, fungal, and AMF richness and community composition were not statistically significant, suggesting that a 0.4 unit increase in soil pH by liming might not be enough for inducing a significant effect on soil microbial community. In contrast, these soil microbes were significantly responsive to land conversion from paddy farms to tea plantations, and the interaction of soil conversion and liming, mechanically underlying by changes in soil physicochemical properties. Subsequently, lime amendment strongly enhanced tea yield and yield components, and the impact was more obvious in the second year following lime application. Our findings contributed towards an understanding of changes in soil biodiversity in response to liming and land conversion and confirm that appropriate liming could be an effective strategy to ameliorate soil acidity, thus enhancing soil biodiversity and crop productivity. Further studies should consider other liming strategies such as application frequency and rates, depth and period, as well as how it affects other organism diversity and function (nematodes, soil microfauna, soil microbial functional diversity) to provide a better understanding of the liming efficiency in enhancing soil food web and soil health of tea plantations.

4. Chapter 4: Conclusion and future perspectives

4.1 Conclusions

Tea is one of the oldest and most popular beverages in the world and is an important crop cultivated in over 50 countries. In Vietnam, tea plants have been cultivated for thousands of years, and played a crucial role in the country's economic development and social stability. Annually, the tea industry creates employment for around 1.5 million people, as well as contributing more than USD 200 million p/a to the national revenue (Bui and Nguyen 2020; Doanh et al. 2018). However, the long-term dominance of conventional tea production which relies strongly on agrochemical inputs has resulted in a range of serious issues, ranging from soil acidification and soil health degradation, decreased quality and production efficiency, as well as human health concerns and environmental problems (Phong et al. 2015a; Van Ho et al. 2019). Additionally, Vietnamese farmers have been converting parts of their allocated land to cultivate tea plants, as tea production could provide a better net income in comparison with other annual crops such as rice and vegetables. However, little is known about how this practice could affect tea soil attributes and plant productivity.

This study was designed to provide a comprehensive overview of tea production in Vietnam, the main challenges of conventional tea farming and the sustainability of agroecological tea management strategy; mechanisms and consequences of tea soil health degradation and soil acidification, as well as to assess the potential uses of agricultural wastes/composts and liming to control soil acidification, thus improving tea soil health- related properties while enhancing crop productivity and quality. In doing so, a total of 66 different tea growing households were selected in the Northern region of Vietnam for the economic efficiency study, and then 20 tea farms from these households were selected for field experiments and sampling. Of the 20 tea plots (10 agroecological plantations and 10 conventional plantations), 10 plots were converted from annual croplands, and 10

were original tea soils. Soil physicochemical properties, soil fauna and root AMF, as well as tea yield and yield components were analyzed to compare the impacts of agroecological and conventional tea management methods. Additionally, the soil bacterial, fungal and AMF community richness and composition were also determined using rDNA and ITS gene sequencing analyses to assess the effect of liming and soil conversion practice on tea soil biodiversity and crop productivity.

Our critical review of the sustainability of agroecological tea management strategy is presented in Chapter 1, which showed that apart from potentially bringing about high productivity in the short term, the continuity of conventional tea production in Vietnam has led to a series of severe issues. Firstly, intensive use of agrochemicals including fertilizers and pesticides has been a very common practice of conventional tea farmers, which led to soil degradation, particularly soil acidification, soil nutrient leaching and imbalance, as well as a high level of soil heavy metals such as Cu, Ni, Zn, Hg, As, Cd, Cr, and pesticide residues (Kundu et al. 2016; Suhag 2016). Inappropriate tea farming practices such as mono cropping, burning or clearing out plant residues and over ploughing have also caused soil erosion, which subsequently facilitated soil nutrient losses and polluted surrounding watercourses and soils (Alam 2014; Sultana et al. 2014; Vezina et al. 2006). In addition, previous investigations have highlighted the negative consequences of extensive pesticide applications in tea cultivation on the environment and health of Vietnamese tea farmers and consumers (Dang et al. 2017; He et al. 2020). Consequently, there has been a growing conversion from conventional tea farming to agroecological tea management practices, which include using organic fertilizers and biofertilizers, mulching and intercropping as well as IPM and IDM. Agroecology relies on the application of natural ecological system processes and concepts for optimizing the interactions between humans, plants, animals and the environment, and can provide a practical way for restoring soil quality depleted by conventional management practices (Altieri et al. 2020; FAO 2020).

In the context of tea production, the beneficial impacts of agroecological management practices on tea soil health-related properties, tea quality indicators and productivity in the long run, as well as human health and the environment in Vietnam have been poorly documented (Doanh et al. 2018; Duc and Goto 2019; Van Ho et al. 2019). Agroecological practices such as the application of organic fertilizers, biofertilizers and biopesticides, organic mulching, intercropping as well as Integrated Pest/Disease Management (IPM/IDM) have been widely known to improve soil physicochemical and biological attributes, mainly due to the additions of organic matter and soil essential macro and micronutrients, which enrich soil organism diversity and functional activities, as well as reduce the use of agrochemical inputs and chemical residues in soil and on tea leaves (Li et al. 2015; Lin et al. 2019; Zhang et al. 2017). Our result also revealed that soil organisms, including soil fauna and microbes have been vital parts of all soil types due to their function in altering and transporting soil components, particularly in organic matter decomposition and soil structure development (Dumanski, 2006; Cardoso et al. 2013; Stoops, 2018). Additionally, soil organisms have widely been considered as an important indicator of soil fertility. Despite tea soil biology being explored for centuries, knowledge of the tea soil flora roles in enhancing tea cultivation is still poorly understood (Dumanski, 2006; Cardoso et al. 2013; Stoops, 2018). Therefore, a better understanding of the role of the soil biological compartment in managing soil fertility in tea tree plantations is obviously essential.

Soil acidification is occurring in about one-third of the world's soils and has been considered as one of the most serious challenges in many tea growing countries, including Vietnam (Huu Chien et al. 2019; Lin et al. 2019; Ni et al. 2018; Zou et al. 2014). In the Chapter 2, we provided a systematic review of the mechanisms and consequences of tea soil acidification, which has mainly been driven by intensive application of mineral nitrogen, and the natural excretion of carbonic acids and polyphenols of tea roots also aggravated the problem (Alekseeva et al. 2011; Ni et al. 2018; Zhang

et al. 2020 (Yan et al. 2018). Soil acidification has numerous consequences on soil chemical and biological properties, as well as tea quality and productivity, in which the reduction and imbalance of nutrient base cations, including Ca^{2+} , Mg^{2+} , Na^+ and K^+ has been considered as one of the most serious disadvantages (Alekseeva et al. 2011; Ni et al. 2018; Zhang et al. 2020). Additionally, strongly acidic soils could lead to an increased accumulation of Al^{3+} and Mn^{2+} , which can inhibit the expansion, elongation and division of root cells, reducing water and nutrient uptake by tea root systems, as well as causing nutrient imbalances, especially with divalent cations such as Mg^{2+} , Zn^{2+} and Ca^{2+} (Alekseeva et al. 2011; Hui et al. 2010). Soil pH is a crucial factor regulating soil organisms, and long-term soil acidification is responsible for depletion of soil organism diversity and functional activities. In more severe cases, up to 70% of important tea soil fauna can be lost if soil pH reduced to below 4.0, and soil enzymatic activities, microbial activities and microbial biomass can be significantly decreased (Han et al. 2007; Li et al. 2017; Zhang et al. 2015). By contrast, soil acidity enhances the environment for growth of some soilborne pathogen diseases, such as *Fusarium oxysporum*, *Fusarium solani* and *Microdidium phyllanthi*, which is the main cause of root rot and die back disease in tea plants (Arafat et al. 2019). When the soil pH is lower than 4.0, tea plant growth is inhibited, affecting both the quality and quantity of tea production (Li et al. 2016; Yan et al. 2020). Moreover, high concentration of Al^{3+} and Mn^{2+} can also negatively affect tea quality indicators such as amino acid composition, reduce the chlorophyll and carotenoid content of tea leaves, and retard tea growth. Serious tea soil acidification can also cause an increase in tea management cost and environmental risks, resulting from annual agricultural production loss and requirements for extensive control methods, as well as the accumulation of chemical metals such as arsenic (As), mercury (Hg), lead (Pb), chromium (Cr), cadmium (Cd) and nickel (Ni) (Bayraklı and Dengiz 2020; Zhang et al. 2020). As agricultural waste and derived products are widely available in the top tea growing countries, this study also provided a critical assessment of how these resources can be utilized to ameliorate tea soil acidification, thus improving tea soil health-related parameters,

and enhancing tea yield and quality indicators. Our findings showed that agricultural waste and by products such as biochar and organic manures have demonstrated a great potential to mitigate soil acidification by tea cultivation due to the natural alkaline characteristics with high pH value and buffering capacity of these materials, which could supply alkaline matter and essential elements to neutralize soil acidity. Apart from mitigating soil acidification, recycling organic amendments as the partial or full substitutes for chemical fertilizers can bring about a range of benefits for other aspects of tea plantation soil health and the environment, such as improving soil OM, soil OC, soil exchangeable cations such as Ca^{2+} , Mg^{2+} , Na^+ and K^+ , and nutrient availability, while reducing risks of Al toxicity, heavy metal accumulation, greenhouse gas emissions and nutrient runoff such as N and P (Cai et al. 2015; He et al. 2019; Lin et al. 2019). The addition of these soil amendments could also enrich soil organisms and ultimately improve tea yield and quality indicators (Bhatt et al. 2019; Cai et al. 2021; Rayne and Aula 2020).

Since tea farmers in Vietnam have rapidly converted their existing conventional tea fields and part of their allocated lands to adopt agroecological tea farming, it is crucial to understand how these practices affect tea plantation soils as well as the quality and quantity of tea production. This thesis aims at providing informative and scientific resources for enabling more informed decisions regarding the management methods, policies and programs to promote agroecological tea management in Vietnam and other tea producing nations. Our investigation is the first comprehensive evaluation of the sustainability of agroecological tea management practices in Northern Vietnam, in comparison with the conventional tea farming system, with a focus on soil health indicators, tea yield and quality, and net income of tea farmers, which is also presented in Chapter 2. We showed that converting conventional tea adoption to agroecological management practices significantly increased tea root AMF intensity by up to 24%, soil macro and mesofauna by 110% and 60%, respectively. Despite this improvement, it is noted that soil faunal community

richness and abundance observed in Northern Vietnam were significantly lower compared to that in previous studies conducted in tea and cropping systems. Organic fertilizers and manure incorporations also significantly reduced soil acidification rates (soil pH increased by 0.5 units on average) because of their naturally alkaline characteristics and provided supplement organic matters, thus improving soil OM, AMF colonization and soil faunal abundance and diversity. In contrast, soil conversion from paddy and other annual crop fields to tea plantations did not lead to any significantly adverse effects on soil health-related attributes, suggesting that this practice could be as effective as cultivating tea in nonconverted lands. Despite the lower tea yields, the agroecological management method led to a significant increase in net income for tea farmers, which was mainly driven by the premium price of agroecological tea products and other credits from supporting agencies. Our findings indicated for the first time that agroecological tea adopters earned around USD 8,400 ha/year more than the farmers still practicing conventional management. Therefore, these practices could be scaled up in Northern Vietnam and other regions, which share similar natural and socioeconomic conditions for more environmentally sustainable economic tea production.

As we have intensively discussed in Chapter 2, tea soil acidification has been a severe issue in many tea growing countries, and Vietnam is not an exception. Our experimental study presented in Chapter 3 demonstrated that despite soil pH being significantly higher in agroecological tea plantations compared with that in tea fields under the conventional management, all the studied tea soils in the region were strongly acidic. Among the options, liming has been considered as the most affordable and practical strategy to reduce soil acidification and hence improve soil health-related properties, in particular soil pH (Holland et al. 2018; Mahmud and Chong 2022; Tunney et al. 2010). To date however, there is still a lack of understanding of the effects of liming on tea soil biodiversity and yield through improving soil health-related properties and how these effects interact with soil

conversion from paddy fields to tea plantations in the world, as well as in Vietnam (Wang et al. 2021). To fill this gap, Chapter 3 explores the impacts of liming and soil conversion on soil chemical properties, soil and organic litter macrofauna, soil microbial communities using 16S rDNA and ITS gene sequencing analyses, and tea yield and yield components. 9 months after the application, liming significantly enhanced soil pH (by 0.4 units) and soil OM, while strongly reduced soil exchangeable Al and Mn, and P availability. Planting tea in newly established tea lands also had higher soil pH values, OM and soil P availability, but also increased soil Al toxicity risk. Macrofauna observed in tea soils were less abundant than in organic mulch materials, and liming also had a significant effect on macrofauna abundance and composition recorded in these layers. The highest average macrofauna intensity (147 individuals/ m²) was observed in mulch materials collected from the converted-lime tea plots, while the lowest figure was recorded in the original non-limed soils (average of 63.9 individuals/ m²), and a total of 11 macrofauna groups were found in both soil and litter layers. Lime amendment also significantly enhanced tea AMF intensity and frequency, as well as tea yield and yield components, regardless of land use history. In contrast, soil bacterial, fungal and AMF relative abundance and composition were strongly responsive to land conversion, and the interaction of liming and soil conversion, mechanically underlying by changes in soil physicochemical properties and the crop types. Sole lime application did not lead to any significant impacts on soil microbial richness nor community composition, indicating that a 0.4-unit shift in soil pH may not be enough to trigger a significant change in soil microbial communities. Additionally, lime incorporation created a better for the growth of some fungal taxa, while suppressing other fungal groups which are preferable to acidic soils, thus fungal diversity appeared to be unaffected by liming. Our findings contribute towards an understanding of changes in soil biodiversity in response to liming and land-use conversion and confirm that appropriate liming could be an effective strategy to ameliorate soil acidity, thus enhancing soil biodiversity and crop productivity.

4.2 Future perspectives

This study provided the first investigation of the sustainability of agroecological tea management adaptation in Northern Vietnam, how lime and agricultural wastes can be utilized to control tea soil acidification and their impacts on soil health indicators, crop productivity and quality. Despite the fact that the adoptions of these farming strategies have resulted in a more economically and environmentally sustainable tea production in Northern Vietnam, our results highlight the complementary research needs as well as supporting programs and policies for better understanding the underlying mechanisms of the impacts of agroecological management practices, soil conversion and liming on soil microbial community diversity and composition, their potential roles in managing and enhancing soil fertility of perennial plantations, and for scaling up agroecological tea production in Northern Vietnam.

The lack of access to inputs required for agroecological practices such as biofertilizers, biopesticides or organic fertilizer, small scale production and lack of understanding of long-term benefits of agroecological tea farming have been identified as the main limiting factors that preventing Vietnamese tea growers from the adoption (Doanh et al. 2018; Tuan 2019; Van Ho et al. 2019). Additionally, limited market information and linkages, especially those involving international markets such as legislation and standards regarding food safety and quality; and the extensive cost for third-party certification processes of organic and VietGAP tea products are among the top challenges (Ha 2014a; Van Ho et al. 2019). These findings underline the importance of further technical and non-technical supporting policies and programs from the governments and other relevant stakeholders, such as promotion of the commercialization of organic fertilizer and biological alternatives for easier accessibility, market information and affordable credit improvement, especially for low-income households (Doanh et al. 2018). In addition, despite agroecological tea method resulted in significant increase in tea root AMF colonization compared

with conventional tea management approach, our results indicated that the highest root mycorrhizal intensity was only 38% across all the trials, suggesting that other options such as application of bioinoculants containing effective AMF should be introduced to improve tea root mycorrhization, and subsequently soil health and plant growth (Bag et al. 2022; Shao et al. 2018).

While promising, the expanding use of agricultural wastes would need further understanding to improve their application efficacy while reducing any potential negative consequences on the environment. First, the risks of introducing heavy metal and pathogens from animal manures, compost and biochar applications have been widely reported (Alegbeleye and Sant'Ana 2020; Dai et al. 2017), but how they could affect soil and tea plants have not been clearly understood. Biochar has been considered as the most expensive soil management solution, particularly for large-scale use in agriculture (Siedt et al. 2020). Additionally, since the majority of findings on positive impacts of biochar in controlling soil acidification have been the outcomes of laboratory or glasshouse studies, these results need to be validated in field conditions (Dai et al. 2017). To date, most studies on utilizing agricultural wastes in tea cultivation have been conducted in China, with specific but limited soil characteristics, climate conditions and tea management practices. Nonetheless, it has been clearly indicated that differences in such conditions could significantly affect the effectiveness of these soil acidification ameliorants (Gu et al. 2019; Siedt et al. 2020; Wu et al. 2020a). This research gap highlights the need and opportunities for further investigations to provide comprehensive knowledge and reliability in recycling these soil amendments.

The present study confirmed that lime application at the rate of 1.5 tons/ha could be an effective solution for reducing soil acidification and subsequently enhancing soil biodiversity and crop productivity. Other application rates and strategies of liming, such as location, application frequency, depth and period, could also have different impacts on soil health indicators, quality and quantity of crop production, thus deserve further investigations (Holland et al. 2018; Li et al. 2019). Moreover,

how lime amendment and land conversion affect soil microbial community structure and function, as well as other groups of soil food webs, including nematodes and other trophic levels of soil fauna, have not been investigated to date. It is also important that we better understand the roles of soil microbial communities in relation to tea quality indicators and other aspects of tea plantation management to ensure that suitable and sustainable management practices are promoted for restoring the soil fertility in the region (Gui et al. 2021; Zhang et al. 2020c). Finally, the strategies developed in this study might also be useful in understanding and improving the sustainable management of other perennial crops in the region, such as coffee and fruit orchards. The conversion from annual crops such as maize and cassava to these perennial crop systems in the region also have been increasingly occurring, but how this practice affects soil biodiversity and crop production efficiency is also largely unknown. Promotion of agroecological farming and other soil acidification management strategies where applicable, could benefit both local population and the environment through a reduction of expensive agrochemical inputs and an increased source of income.

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Appendix 1

AUTHORSHIP STATEMENT- CHAPTER ONE

Details of publication and executive author

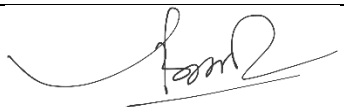
Title of Publication		Publication details
Sustainable tea production through agroecological management practices in Vietnam: a review		Environmental Sustainability 4, 589–604 (2021). https://doi.org/10.1007/s42398-021-00182-w
Name of executive author	School/Institute/Division if based at Deakin; Organisation and address if non-Deakin	Email or phone
Viet San Le	School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	sanl@deakin.edu.au

2. Inclusion of publication in a thesis

Is it intended to include this publication in a higher degree by research (HDR) thesis?	Yes / No	If Yes, please complete Section 3 If No, go straight to Section 4.
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3. HDR thesis author’s declaration

Name of HDR thesis author if different from above. (If the same, write “as above”)	School/Institute/Division if based at Deakin	Thesis title
As above	School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	Agroecological practices for a sustainable tea production in Northern Vietnam
If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)		

<i>I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.</i>	Signature and date	 10/1/2023
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4. Description of all author contributions

Name and affiliation of author	Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)
Dr. Didier Lesueur Centre de Cooperation Internationale en Recherche, Agronomique pour le Developpement (CIRAD), UMR Eco&Sols, Hanoi, Vietnam	Drafting of manuscript, expert guidance and critique
Dr. Laetitia Herrmann Alliance of Bioversity International and International Center for Tropical Agriculture (CIAT), Asia hub, Common Microbial Biotechnology Platform (CMBP), Hanoi, Vietnam	Drafting of manuscript, expert guidance and critique
Dr. Lee Hudek School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	Drafting of manuscript, expert guidance and critique
Dr. Luu Ngoc Quyen The Northern Mountainous Agriculture and Forestry Science Institute (NOMAFSI), Phu Tho, Vietnam	Expert guidance and critique
A/Prof. Lambert Bräu School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	Drafting of manuscript, expert guidance and critique

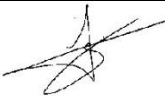

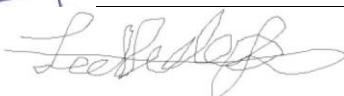

5. Author Declarations

I agree to be named as one of the authors of this work, and confirm:

- i. that I have met the authorship criteria set out in the Deakin University Research Conduct Policy,*
- ii. that there are no other authors according to these criteria,*
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Dr. Didier Lesueur		25/2/2023
Dr. Laetitia Herrmann		25/2/2023
Dr. Lee Hudek		3/3/2023
Dr. Luu Ngoc Quyen		10/2/2023
A/Prof. Lambert Bräu		5/3/2023

6. Other contributor declarations

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Name and affiliation of contributor	Contribution	Signature* and date

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Appendix 2

AUTHORSHIP STATEMENT- CHAPTER TWO

1. Details of publication and executive author

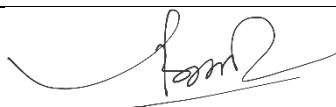
Title of Publication		Publication details
How application of agricultural waste can enhance soil health in soils acidified by tea cultivation: a review		Environ Chem Lett 20, 813–839 (2022). https://doi.org/10.1007/s10311-021-01313-9
Name of executive author	School/Institute/Division if based at Deakin; Organisation and address if non-Deakin	Email or phone
Viet San Le	School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	sanl@deakin.edu.au

2. Inclusion of publication in a thesis

Is it intended to include this publication in a higher degree by research (HDR) thesis?	Yes / No	If Yes, please complete Section 3 If No, go straight to Section 4.
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3. HDR thesis author’s declaration

Name of HDR thesis author if different from above. (If the same, write “as above”)	School/Institute/Division if based at Deakin	Thesis title
As above	School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	Agroecological practices for a sustainable tea production in Northern Vietnam
If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)		

<i>I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.</i>	Signature and date	 16/1/2023

4. Description of all author contributions

Name and affiliation of author	Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)
Dr. Didier Lesueur Centre de Cooperation Internationale en Recherche, Agronomique pour le Developpement (CIRAD), UMR Eco&Sols, Hanoi, Vietnam	Drafting of manuscript, expert guidance and critique
Dr. Laetitia Herrmann Alliance of Bioversity International and International Center for Tropical Agriculture (CIAT), Asia hub, Common Microbial Biotechnology Platform (CMBP), Hanoi, Vietnam	Drafting of manuscript, expert guidance and critique
Dr. Lee Hudek School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	Drafting of manuscript, expert guidance and critique
Msc Thi Binh Nguyen Independent researcher	Drafting of manuscript and revision
A/Prof. Lambert Bräu School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	Drafting of manuscript, expert guidance and critique

5. Author Declarations


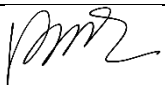

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iv. that the data on which these findings are based are stored as set out in Section 7 below.

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Name of author	Signature*	Date
Dr. Didier Lesueur		25/2/2023
Dr. Laetitia Herrmann		25/2/2023
Dr. Lee Hudek		5/3/2023
Msc Thi Binh Nguyen		16/1/2023
A/Prof. Lambert Bräu		10/3/2023

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Name and affiliation of contributor	Contribution	Signature* and date

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Appendix 3

AUTHORSHIP STATEMENT- CHAPTER TWO

1. Details of publication and executive author


Title of Publication		Publication details
Sustainable green tea production through agroecological management and land conversion practices for restoring soil health, crop productivity and economic efficiency: Evidence from Northern Vietnam		Soil Use Manage. 2023; 00:1–20. https://doi.org/10.1111/sum.12885
Name of executive author	School/Institute/Division if based at Deakin; Organisation and address if non-Deakin	Email or phone
Viet San Le	School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	sanl@deakin.edu.au

2. Inclusion of publication in a thesis

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3. HDR thesis author’s declaration

Name of HDR thesis author if different from above. (If the same, write “as above”)	School/Institute/Division if based at Deakin	Thesis title
As above	School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	Agroecological practices for a sustainable tea production in Northern Vietnam
If there are multiple authors, give a full description of HDR thesis author’s contribution to the publication (for example, how much did you contribute to the conception of the project, the design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)		

<i>I declare that the above is an accurate description of my contribution to this paper, and the contributions of other authors are as described below.</i>	Signature and date	 16/1/2023
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4. Description of all author contributions

Name and affiliation of author	Contribution(s) (for example, conception of the project, design of methodology or experimental protocol, data collection, analysis, drafting the manuscript, revising it critically for important intellectual content, etc.)
Dr. Didier Lesueur Centre de Cooperation Internationale en Recherche, Agronomique pour le Developpement (CIRAD), UMR Eco&Sols, Hanoi, Vietnam	Drafting of manuscript, expert guidance and critique
Dr. Laetitia Herrmann Alliance of Bioversity International and International Center for Tropical Agriculture (CIAT), Asia hub, Common Microbial Biotechnology Platform (CMBP), Hanoi, Vietnam	Drafting of manuscript, expert guidance and critique
A/Prof. Lambert Bräu School of Life and Environmental Sciences, Faculty of Science, Engineering and Built Environment–Deakin University, Melbourne, VIC 3125, Australia	Drafting of manuscript, critique and revision

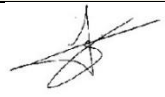

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Name of author	Signature*	Date
Dr. Didier Lesueur		25/2/2023
Dr. Laetitia Herrmann		25/2/2023

A/Prof. Lambert Bräu		10/3/2023
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Appendix 4

Supplementary Table S1. Description of management practices of the studied agroecological tea plantations.

Plantations	Coordinates	Fertilization	Mulching practices	Pet and disease control	Land use history
AO1	21°32'26.45" N 105° 45'46" E Elevation: 40m Slope: 11°	- Organic compost: Buffalo, cow and chicken manure mixed with soybean and/or fish power, applied 3.5 tons/ha/year during the pruning season. - Commercial organic fertilizer applied 300 kg/ha/harvest (Green Cao Nguyen 01: OM 24 %, P ₂ O ₅ 2.4 %; K ₂ O 1.1 %, total N 2.5%; pH 5; moisture 30 %; Tien Nong: OM 23 %, humic acid 2.5 %, total N 2.5 %; pH 5; moisture 20 %). - Bioorganic fertilizer: Trichoderma: Trichoderma spp (in 1kg): 1.106 CFU/g, OM 23 %, total N 2.5%; pH 6; moisture 20 %.	Mulching by using organic materials (mainly Fern (Gleichenia linearis), 30 cm thick and other plant residues), applied since the first year of tea plantation. Mulches were applied one per year, after the pruning.	- BT-EMI (Japan, Bacillus Thuringiensis 1.3 x 10 ⁸ CFU/ml) - Neem Nin (India; Azadirachtin 0.3% w/w) - ANISAF SH01(Vietnam, Polyphenol 20g/l) - Manual control: Insect traps, herbs, predators, field sanitation, plucking.	Land has been using for tea cultivation for more than 30 years. Before that time, land was used for commercial forests.
AO2	21°32'31.53" N 105° 45'50" E Elevation: 45m Slope: 12°	- Organic manure: Buffalo and chicken compost, applied 3.5-4 tons/ha/year during the pruning season. - Commercial organic fertilizer applied 300kg/ha/harvest (Green Cao Nguyen 01: OM 24 %, P ₂ O ₅ 2.4 %; K ₂ O 1.1 %, total N 2.5 %; pH 5; moisture 30%; Tien Nong: OM 23 %, humic acid 2.5 %, total N 2.5 %; pH 5; moisture 20%). - Bioorganic fertilizer: Trichoderma 1kg: Trichoderma spp (in 1 kg): 1.10 ⁶ CFU/g OM 23 %, total N 2.5 %; pH 6; moisture 20 %.	Mulching by using organic materials (mainly Acacia and eucalyptus barks and residues, 20 cm thick. Mulches were applied every 2 years since the first year of tea plantation, after the tea pruning.	- BT-EMI (Japan, Bacillus thuringiensis 1.3 x 10 ⁸ CFU/ml) - Neem Nin (India; Azadirachtin 0.3 % w/w) - ANISAF SH01(Vietnam, Polyphenol 20 g/l) - Manual control: Insect traps, herbs, predators, field sanitation, plucking.	Land has been using for tea cultivation for more than 20 years. Before that time, land was used for commercial Acacia
AO3	21°32'28" N 105° 45'35" E Elevation: 40m Slope: 12°	- Organic compost: Buffalo and cow manure, applied 3.5 tons/ha/year during the pruning season. - Commercial organic fertilizer applied 250 kg/ha/harvest (Green Cao Nguyen 01: OM 24 %, P ₂ O ₅ 2.4 %; K ₂ O 1.1 %, total N 2.5 %; pH	Mulching by using organic materials (mainly Fern, 30cm thick and other plant residues), applied since the first year of tea plantation.	- BT-EMI (Japan, Bacillus thuringiensis 1.3 x 10 ⁸ CFU/ml) - ANISAF SH01(Vietnam, Polyphenol 20 g/l)	Land has been using for tea cultivation for more than 25 years. Before that time, land was used for

		5; moisture 30 %; T159 Gold Plus: OM 40 %, humic acid 2 %, fulvic acid 1.2%, C/N 12; pH 6.5; moisture 30 %, macronutrients: Ca, Mg, S, SiO \geq 1%; micronutrients: Fe, Cu, Mn, Co, Bo: 100ppm).	Mulches were applied one per year, after the pruning.	- Manual control: Insect traps, herbs, predators, field sanitation, plucking.	commercial Acacia
AO4	21°33'42.02" N 105° 45'28.79" E Elevation: 40m Slope: 14°	- Organic compost: Buffalo and cow manure, applied 3.5 tons/ha/year during the pruning season. - Commercial organic fertilizer applied 300kg/ha/harvest (Green Cao Nguyen 01: OM 24 %, P ₂ O ₅ 2.4 %; K ₂ O 1.1%, total N 2.5 %; pH 5; moisture 30 %; Tien Nong: OM 23 %, humic acid 2.5%, total N 2.5 %; pH 5; moisture 20 %). - Bioorganic fertilizer: Trichoderma: Trichoderma spp (in 1kg): 1.10 ⁶ CFU/g OM 23 %, total N 2.5 %; pH 6; moisture 20 %.	Mulching by using organic materials (mainly Acacia and eucalyptus residues, 20cm thick. Mulches were applied every 2 years since the first year of tea plantation, after the tea pruning.	- BT-EMI (Japan, Bacillus thuringiensis 1.3 x 10 ⁸ CFU/ml) - Neem Nin (India; Azadirachtin 0.3 % w/w) - ANISAF SH01(Vietnam, Polyphenol 20 g/l) - Manual control: Insect traps, herbs, field sanitation, plucking.	Land has been using for tea cultivation for more than 30 years. Before that time, land was used for planting Acacia
AO5	21°32'16" N 105° 45'26" E Elevation: 45m Slope: 11°	- Organic compost: Buffalo and cow manure, applied 3.5 tons/ha/year during the pruning season. - Commercial organic fertilizer applied 300 kg/ha/harvest (Green Cao Nguyen 01: OM 24 %, P ₂ O ₅ 2.4 %; K ₂ O 1.1%, total N 2.5 %; pH 5; moisture 30 %; T159 Gold Plus: OM 40 %, humic acid 2 %, fulvic acid 1.2 %, C/N 12; pH 6.5; moisture 30%, macronutrients: Ca, Mg, S, SiO \geq 1%; micronutrients: Fe, Cu, Mn, Co, Bo: 100ppm).	Mulching by using organic materials (mainly Fern, 30cm thick and other plant residues), applied since the first year of tea plantation. Mulches were applied one per year, after the pruning.	- BT-EMI (Japan, Bacillus thuringiensis 1.3 x 10 ⁸ CFU/ml) - Neem Nin (India; Azadirachtin 0.3 % w/w) - ANISAF SH01(Vietnam, Polyphenol 20 g/l) - Manual control: Insect traps, herbs, field sanitation, plucking.	Land has been using for tea cultivation for more than 30 years. Before that time, land was used for planting commercial forests
AC1	21°32'47.09" N 105° 45'41.38" E Elevation: 30m Slope: 6°	- Organic compost: Buffalo, cow and chicken manure mixed with soybean and/or fish power, applied 3.5-4 tons/ha/year during the pruning season. - Commercial organic fertilizer applied 300 kg/ha/harvest (Green Cao Nguyen 01: OM 24 %, P ₂ O ₅ 2.4 %; K ₂ O 1.1 %, total N 2.5 %; pH 5; moisture 30 %) - Bioorganic fertilizer: Trichoderma 1kg;	Mulching by using organic Acacia and Eucalyptus residues, 20cm thick and other plant residues), applied since the first year of tea plantation. Mulches were applied in every 2 years, after the tea pruning	- BT-EMI (Japan, Bacillus thuringiensis 1.3 x 10 ⁸ CFU/ml) - Neem Nin (India; Azadirachtin 0.3 % w/w) - ANISAF SH01(Vietnam, Polyphenol 20 g/l) - Manual control: Insect	Tea has been in its first generation (6 years old). Before tea cultivation, land was used as paddy, with one rice growing season per year.

		Trichoderma spp: 1.10 ⁶ CFU/g, OM 23 %, total N 2.5 %; pH 6; moisture 20 %.	season.	traps, herbs, field sanitation, plucking.	
AC2	21°32'24" N 105° 45'49.60" E Elevation: 35m Slope: 8°	- Organic compost: Buffalo and cow manure, applied 3-4 tons/ha/year during the pruning season. - Commercial organic fertilizer applied 250 kg/ha/harvest (T159 Gold Plus: OM 40 %, humic acid 2 %, fulvic acid 1.2%, C/N 12; pH 6.5; moisture 30 %, macronutrients: Ca, Mg, S, SiO ≥ 1%; micronutrients: Fe, Cu, Mn, Co, Bo: 100 ppm).	Mulching by using organic Acacia and Eucalyptus residues, 20cm thick and other plant residues), applied since the first year of tea plantation. Mulches were applied in every 2 years, after the tea pruning season.	- Neem Nin (India; Azadirachtin 0.3 % w/w) - ANISAF SH01 (Vietnam, Polyphenol 20 g/l) - Manual control: Insect traps, herbs, field sanitation, plucking.	Tea has been in its first generation (6 years old). Before tea cultivation, land was used for one rice season and vegetables and maize.
AC3	21°32'22" N 105°44'32" E Elevation: 30m Slope: 9°	- Organic compost: Buffalo, cow and chicken manure mixed with soybean and/or fish power, applied 3.5 tons/ha/year during the pruning season. - Commercial organic fertilizer applied 300 kg/ha/harvest (Green Cao Nguyen 01: OM 24 %, P ₂ O ₅ 2.4 %; K ₂ O 1.1 %, total N 2.5 %; pH 5; moisture 30 %; Tien Nong: OM 23%, humic acid 2.5 %, total N 2.5%; pH 5; moisture 20 %). - Bioorganic fertilizer: Trichoderma: Trichoderma spp (in 1kg): 1.10 ⁶ CFU/g, OM 23 %, total N 2.5 %; pH 6; moisture 20 %.	Mulching by using rice straw, 20cm thick and other plant residues), applied since the first year of tea plantation. Mulches were applied in every year, after the tea pruning season	- BT-EMI (Japan, Bacillus thuringiensis 1.3 x 10 ⁸ CFU/ml) - Neem Nin (India; Azadirachtin 0.3 % w/w) - ANISAF SH01 (Vietnam, Polyphenol 20 g/l) - Manual control: Insect traps, herbs, field sanitation, plucking.	Tea has been in its first generation (6 years old). Before tea cultivation, land was used for cultivating vegetables and maize.
AC4	21°32'37.48" N 105°45'53.40" E Elevation: 30m Slope: 8°	- Organic compost: Pig and chicken manure mixed with biogas wastewater, applied 4 tons/ha/year during the pruning season. - Commercial organic fertilizer applied 300 kg/ha/harvest (T159 Gold Plus: OM 40 %, humic acid 2 %, fulvic acid 1.2%, C/N 12; pH 6.5; moisture 30%, macronutrients: Ca, Mg, S, SiO ≥ 1 %; micronutrients: Fe, Cu, Mn, Co, Bo: 100ppm).	Mulching by using organic Acacia and Eucalyptus residues, 20cm thick and other plant residues), applied since the first year of tea plantation. Mulches were applied in every 2 years, after the tea pruning	- BT-EMI (Japan, Bacillus thuringiensis 1.3 x 10 ⁸ CFU/ml) - ANISAF SH01(Vietnam, Polyphenol 20 g/l) - Manual control: Insect traps, herbs, field sanitation, plucking.	Land has been used for planting tea for 6 years. Before tea cultivation, land was used for cultivating vegetables and maize.

			season.		
AC5	21°34'29.63" N 105° 47' 8.53" E E: 35m Slope: 7°	- Commercial organic fertilizer applied 300kg/ha/harvest (T159 Gold Plus: OM 40 %, humic acid 2 %, fulvic acid 1.2 %, C/N 12; pH 6.5; moisture 30 %, macronutrients: Ca, Mg, S, SiO ≥ 1 %; micronutrients: Fe, Cu, Mn, Co, Bo: 100ppm).	Mulching by using Fern plants, 30cm thick and other plant residues), applied since the first year of tea plantation. Mulches were applied in every 1.5 years, after the tea pruning season	- BT-EMI (Japan, Bacillus thuringiensis 1.3 x 10 ⁸ CFU/ml) - ANISAF SH01 (Vietnam, Polyphenol 20 g/l) - Manual control: Insect traps, herbs, field sanitation, plucking.	Land has bene used for planting tea for 6 years. Before tea cultivation, land was used for one rice season.

Supplementary Table S2. Description of management practices of the studied conventional tea plantations.

Plantations	Coordinates	Fertilization	Mulching practices	Pet and disease control	Land use history
CO1, CO2, CO3, CO4, CO5	21°32' - 21°35' N 105° 45' - 105° 47' E Elevation: 40-50m Slope: 10-15°	Chemical fertilizers (NPK compound and single nitrogen) as the main fertilizer source. Lam Thao NPK 16-8-8: 3- 3.5 tons/ha/year, Urea 150- 200 kg/ha/harvest. Organic fertilizers: Less than 1 ton/ha/year.	No mulching practices in the last 5 years.	Using chemical pesticides (e.g., active ingredients: Imibenconazole, Mancozeb, Bup rofezin, Acrinathrin, Etofenprox) as the main pest and disease management.	Land has been using for tea cultivation for more than 30 years. Before that time, land was used for commercial forests.
CC1, CC2, CC3, CC4, CC5	21°32' - 21°35' N 105° 45' - 105° 47' E Elevation: 35- 45m Slope: 6-10°	Chemical fertilizers (NPK compound and single nitrogen) as the main fertilizer source. Lam Thao NPK 16-8-8: 3- 3.5 tons/ha/year, Urea 100- 150kg/ha/harvest. Organic fertilizers: Less than 1 ton/ha/year.	No mulching practices in the last 5 years	Using chemical pesticides (e.g., active ingredients: Imibenconazole, Mancozeb, Bup rofezin, Acrinathrin, Etofenprox) as the main pest and disease management.	Land has bene used for planting for 6 years. Before tea cultivation, land was used for annual crops such as one rice season, vegetables and maize.

Supplementary Table S3. Two-way analyses of variance (ANOVA) results for the impact of cultivation management, soil conversion and their interaction on soil physical and chemical properties. Results in boldface are statistically different ($P < 0.05$)

Treatment/indicators	pH (H ₂ O)		OM (%)		P (mg/100g)		Total N (%)		Sand		Silk		Clay	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Cultivation practice	6.86	0.002	25.0	<0.0001	1.211	0.306	2.565	0.086	4.046	0.053	0.206	0.815	2.404	0.100
Land type	1.18	0.28	0.001	0.972	0.333	0.566	1.409	0.240	2.364	0.130	1.678	0.200	0.030	0.863
Cultivation x Land type	4.26	0.009	18.115	<0.0001	1.046	0.380	3.305	0.027	3.837	0.066	1.202	0.317	1.335	0.272

Supplementary Table S4. Two-way analyses of variance (ANOVA) results for the impact of cultivation management, soil conversion and their interaction on diversity indexes of soil macro and mesofauna. Results in boldface are statistically different ($P < 0.05$)

Source	Soil macrofauna ($\geq 2\text{mm}$)						Soil mesofauna ($< 2\text{mm}$)											
	Abundance		Richness		Shannon index		Centrifuge extraction						Funnel extraction					
							Abundance		Richness		Shannon index		Abundance		Richness		Shannon index	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Cultivation practice	33.86	<0.0001	5.10	0.018	12.01	0.0165	68.32	<0.0001	28.44	<0.0001	29.75	<0.0001	69.57	<0.0001	12.06	0.003	7.43	0.015
Land type	1.183	0.292	1.20	0.288	3.573	0.075	0.282	0.602	0.01	0.903	1.71	0.209	1.35	0.261	0.98	0.336	1.11	0.307
Cultivation x Land type	0.14	0.706	0.40	0.531	0.86	0.367	0.95	0.344	0.75	0.398	2.79	0.114	0.95	0.342	0.24	0.627	0.71	0.411

Supplementary Table S5. Densities (ind./1m²) of soil macrofauna taxonomic groups recorded in different tea plantations (mean ± SD)

Groups	Funnel extraction				Centrifuging extraction			
	AO	AC	CO	CC	AO	AC	CO	CC
Oribatei	13 ± 1.42a	17 ± 2.21a	11 ± 0.89a	16 ± 1.41a	21 ± 2.51a	25 ± 5.14a	18 ± 2.51a	33 ± 4.93a
Springtails	8 ± 1.15a	12 ± 2.17a	6 ± 1.64a	4 ± 1.34a	11 ± 2.16a	9 ± 2.5a	8 ± 2.07a	1 ± 0.44a
Spider	6 ± 1.64a	8 ± 0.64a	4 ± 1.34a	3 ± 1.15a	3 ± 2.68a	6 ± 1.64a	3 ± 1.34a	3 ± 1.29a
Fly	6 ± 1.61a	4 ± 1.34a	6 ± 1.64a	2 ± 0.22a	4 ± 1.63a	6 ± 1.61a	3 ± 1.32a	3 ± 1.32a
Beetle	10 ± 2.36a	7 ± 1.64a	5 ± 1.34a	4 ± 1.34a	8 ± 1.60a	9 ± 2.30a	2 ± 0.39a	4 ± 0.39a
Pseudoscorpions	5 ± 1.64a	4 ± 1.51a	2 ± 0.2a	4 ± 1.39a	6 ± 1.61a	5 ± 1.34a	6 ± 0.52a	2 ± 0.89a
Polidesmidae	3 ± 1.33a	5 ± 1.68a	5 ± 0.89a	4 ± 0.14a	5 ± 1.57a	7 ± 1.64a	3 ± 0.54a	2 ± 0.22a
Enchytraeids	6 ± 1.64a	5 ± 1.64a	4 ± 1.30a	3 ± 0.34a	8 ± 2.49a	12 ± 4.08a	3 ± 1.34a	3 ± 0.81a
Millipede	11 ± 1.64a	15 ± 2.12a	5 ± 0.89b	8 ± 1.3ab	5 ± 2.51b	16 ± 1.30a	7 ± 1.51b	9 ± 1.64b
Diptera	8 ± 3.91a	10 ± 1.57a	3 ± 1.34a	5 ± 0.3a	4 ± 2.30a	9 ± 1.61a	2 ± 0.54a	0 ± 0a
Mites	4 ± 1.64a	5 ± 1.34a	2 ± 0.14a	4 ± 0.89a	5 ± 1.41a	6 ± 1.34a	1 ± 0.44a	3 ± 0.62a
Ant	6 ± 1.95a	5 ± 1.34a	3 ± 0.1a	4 ± 0.44a	5 ± 2.16a	10 ± 2.44a	4 ± 1.33a	3 ± 0.89a
Mosquito	6 ± 1.64a	5 ± 1.64a	4 ± 1.30a	5 ± 1.34a	6 ± 1.31a	9 ± 2.16a	3 ± 1.34a	1 ± 0.41a
<i>Abundance</i>	<i>80 ± 8.23ab</i>	<i>101 ± 7.11a</i>	<i>58 ± 3.78ab</i>	<i>68 ± 4.77b</i>	<i>92 ± 6.08a</i>	<i>129 ± 4.06a</i>	<i>63 ± 2.51b</i>	<i>65 ± 2.00b</i>
<i>Richness</i>	<i>6.8 ± 1.82a</i>	<i>6.4 ± 0.89ab</i>	<i>4.4 ± 1.51ab</i>	<i>3.2 ± 1.30b</i>	<i>7.4 ± 1.55a</i>	<i>8 ± 1.87ab</i>	<i>3.8 ± 0.83b</i>	<i>3 ± 1.41b</i>
<i>Shannon index</i>	<i>1.73 ± 0.51a</i>	<i>1.69 ± 0.27b</i>	<i>1.36 ± 0.40ab</i>	<i>0.97 ± 0.55b</i>	<i>1.84 ± 0.41ab</i>	<i>1.89 ± 0.27a</i>	<i>1.25 ± 0.10bc</i>	<i>0.8 ± 0.45c</i>

Note: Values followed by different letters are significantly different at $P < 0.05$, according to the Tukey (HSD) tests

Supplementary Table S6. Density (ind./100 g fresh soil) of soil mesofauna taxonomic groups recorded in different managed tea plantations, using the funnel and centrifuging methods (mean \pm SD).

Groups	Agroecological original (AO)	Agroecological converted (AC)	Conventional original (CO)	Conventional converted (CC)
Earthworm	24.6 \pm 8.62a	18.8 \pm 5.67a	10.6 \pm 1.67ab	4.4 \pm 2.46b
Centipede	10.8 \pm 4.08b	24.4 \pm 4.42a	4.2 \pm 2.19b	10.2 \pm 1.92b
Spider	6 \pm 2.23a	4.6 \pm 2.19ab	1 \pm 1.00b	2 \pm 1.22b
Millipede	4 \pm 1.58ab	11.4 \pm 3.12a	1.6 \pm 1.34b	6 \pm 2.23ab
Insect larvae	3.2 \pm 2.28a	3.8 \pm 1.93a	2.4 \pm 1.67a	2.8 \pm 1.92a
Springtails	5 \pm 1.86a	6 \pm 1.93a	2.8 \pm 1.31a	1.8 \pm 0.83a
Ant	14.8 \pm 5.04a	16.6 \pm 7.05a	10.2 \pm 3.12a	8.9 \pm 1.23a
Termite	4.6 \pm 1.87a	7.2 \pm 3.42a	5.8 \pm 1.91a	5 \pm 1.73a
<i>Abundance</i>	68.4 \pm 9.2ab	85.6 \pm 8.49a	32.8 \pm 8.44b	37.48 \pm 8.10b
<i>Richness</i>	7.2 \pm 0.81a	6.8 \pm 1.32ab	3.8 \pm 0.77b	4.4 \pm 1.35b
<i>Shannon index</i>	1.41 \pm 0.11ab	1.37 \pm 0.17a	0.87 \pm 0.14b	0.90 \pm 0.12b

Note: Values followed by different letters are significantly different at $P < 0.05$, according to the Tukey (HSD) tests

Appendix 5

Supplementary Table S7. Two- way analysis of variance (ANOVA) test results for the impact of liming, land type (converted from paddy field vs original tea plantation) and their interaction on diversity indices of soil chemical properties. Results in bold are statistically different ($P < 0.05$).

Treatment/indicators	pH (H ₂ O)		OM (%)		Olsen P (mg/100g)		Total N (%)		Al ³⁺ (Cmol/kg)		Mn ²⁺ (Cmol/kg)	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Liming	143.95	<0.0001	6.96	0.010	8.45	0.036	0.42	0.517	13.08	<0.0001	69.61	<0.0001
Land type	16.00	<0.0001	0.13	0.718	0.86	0.354	0.03	0.852	61.50	<0.0001	0.07	0.793
Liming x Land type	0.08	0.992	1.33	0.250	0.05	0.752	4.94	0.029	5.45	0.022	3.51	0.064

Supplementary Table S8. Analysis of variance (ANOVA) test results for the impact of liming, land type (converted from paddy field vs original tea plantation) on macrofauna observed in mulch and soil materials. Results in boldface are statistically different ($P < 0.05$)

Treatment/indices	Soil macrofauna						Mulch macrofauna					
	Abundance		Richness		Shannon		Abundance		Richness		Shannon	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Liming (original land)	15.89	<0.0001	16.05	<0.0001	9.25	0.005	15.76	<0.0001	0.79	0.379	0.03	0.845
Liming (converted land)	3.625	0.065	5.54	0.024	3.28	0.079	7.550	0.010	19.19	<0.0001	16.88	<0.0001
Liming (all lands)	15.48	<0.0001	19.72	<0.0001	11.02	0.001	19.92	<0.0001	14.05	<0.0001	8.60	0.005
Land type	5.16	0.026	0.33	0.564	0.82	0.367	3.856	0.054	1.14	0.288	0.09	0.765
Liming x Land type	0.83	0.365	0.93	0.338	0.25	0.618	0.022	0.882	6.24	0.015	7.00	0.010

Supplementary Table S9. Effects of liming on the abundance of macrofauna of different taxonomic groups (individuals/m²) in different land use history (original and converted) and materials (soil and mulch) (mean \pm SD). For a given taxonomic group, within each material treatment, values followed by different letters are significantly different at $P < 0.05$, according to the Tukey (HSD) tests.

Groups	Soil				Mulch			
	Original land		Converted land		Original land		Converted land	
	Control	Lime	Control	Lime	Control	Lime	Control	Lime
Earthworm	26.12 \pm 4.01a	35.00 \pm 4.85b	26.67 \pm 5.14a	36.80 \pm 6.60b	23.3 \pm 3.67a	19.7 \pm 2.12a	16.4 \pm 1.89a	23.6 \pm 3.21a
Millipede	16.6 \pm 2.42ab	13.6 \pm 2.36a	31.38 \pm 3.68c	22.7 \pm 3.05b	26.8 \pm 3.32a	22.4 \pm 3.14a	56.3 \pm 5.56b	45.7 \pm 5.21b
Centipede	2.5 \pm 0.31a	5.3 \pm 0.42b	3.8 \pm 0.45ab	6.9 \pm 0.58b	9.72 \pm 1.12a	20.33 \pm 2.45ab	14.44 \pm 1.65ab	25.83 \pm 3.16b
Spider	1.39 \pm 0.42a	1.94 \pm 0.27a	2.22 \pm 2.42a	3.34 \pm 2.42a	1.32 \pm 0.27a	1.11 \pm 0.16a	1.12 \pm 0.14a	1.68 \pm 0.17a
Insect larvae	4.44 \pm 0.51a	5.56 \pm 0.55a	2.9 \pm 0.37a	5.00 \pm 0.52a	0.83 \pm 0.009a	2.22 \pm 0.31a	0.86 \pm 0.01a	2.79 \pm 0.32a
Springtails	2.22 \pm 0.28a	4.45 \pm 0.61a	1.92 \pm 0.24a	4.45 \pm 0.55a	3.05 \pm 0.43a	2.39 \pm 0.32a	2.11 \pm 0.28a	3.05 \pm 0.39a
Enchytraeids	7.22 \pm 0.81a	10.00 \pm 1.11a	7.50 \pm 0.71a	7.79 \pm 0.89a	4.54 \pm 0.51a	21.38 \pm 2.94b	4.89 \pm 0.54a	11.68 \pm 1.22ab
Beetle	1.39 \pm 0.15ab	5.28 \pm 0.32b	0.83 \pm 0.11a	3.89 \pm 0.41ab	3.16 \pm 0.52a	3.55 \pm 0.67a	1.11 \pm 0.19a	2.22 \pm 0.29a
Ant	0.83 \pm 0.09a	2.22 \pm 0.26a	1.12 \pm 0.18a	1.94 \pm 0.21a	10.8 \pm 1.16b	10.2 \pm 1.24b	1.3 \pm 0.15a	8.33 \pm 0.91b
Termite	4.83 \pm 0.45b	2.00 \pm 0.57a	5.56 \pm 0.58b	2.72 \pm 0.54a	4.39 \pm 0.46b	2.44 \pm 0.57a	5.79 \pm 0.62b	1.33 \pm 0.46a
Snails	0.83 \pm 0.12a	2.22 \pm 0.26a	1.11 \pm 0.24a	2.22 \pm 0.26a	1.38 \pm 0.12a	2.78 \pm 0.31a	2.90 \pm 0.35a	8.61 \pm 0.94b

Appendix 6

Supplementary Table S10. Correlation between soil macrofauna species with soil chemical indicators, according to the Pearson test. Values in bold are different from 0 with a significance level $\alpha = 0.05$.

Variables	pH	OM	N	Osen_P	AB+	Mn2+	Earthworms	Millipede	Centipede	Spider	Insect_larve	Springtails	Enchytraeids	Bettle	Ant	Termite	Snails
pH	1	0.103	0.243	0.342	-0.144	0.024	0.263	0.099	0.058	0.130	0.027	0.172	0.039	0.227	0.049	0.269	0.291
OM	0.103	1	0.507	0.039	-0.145	0.018	-0.184	-0.218	0.029	-0.062	0.009	-0.035	-0.111	0.424	0.157	0.142	0.049
N	0.243	0.507	1	0.189	-0.064	0.146	-0.030	0.031	0.194	-0.116	0.054	-0.054	-0.029	0.277	0.070	0.193	0.078
Osen_P	0.342	0.039	0.189	1	0.207	0.071	0.349	-0.092	0.037	0.145	-0.126	0.088	0.209	0.192	0.208	0.110	0.089
AB+	-0.144	-0.145	-0.064	0.207	1	0.073	-0.087	0.259	-0.028	0.095	-0.176	-0.133	0.100	-0.201	-0.140	-0.051	0.023
Mn2+	0.024	0.018	0.146	0.071	0.073	1	-0.025	0.181	0.018	-0.062	-0.124	0.132	-0.038	0.021	-0.074	-0.197	0.017
Earthworms	0.263	-0.184	-0.030	0.349	-0.087	-0.025	1	-0.127	-0.015	0.246	0.123	-0.052	0.136	0.073	0.094	0.074	-0.074
Millipede	0.099	-0.218	0.031	-0.092	0.259	0.181	-0.127	1	0.105	0.059	-0.082	0.127	-0.096	-0.150	0.010	-0.245	-0.056
Centipede	0.058	0.029	0.194	0.037	-0.028	0.018	-0.015	0.105	1	-0.033	-0.021	-0.042	-0.186	0.131	0.215	-0.046	-0.112
Spider	0.130	-0.062	-0.116	0.145	0.095	-0.062	0.246	0.059	-0.033	1	0.070	0.022	0.044	-0.080	0.112	-0.145	-0.025
Insect_larve	0.027	0.009	0.054	-0.126	-0.176	-0.124	0.123	-0.082	-0.021	0.070	1	-0.079	0.119	-0.047	0.008	0.001	0.007
Springtails	0.172	-0.035	-0.054	0.088	-0.133	0.132	-0.052	0.127	-0.042	0.022	-0.079	1	0.045	0.090	0.001	0.015	-0.118
Enchytraeids	0.039	-0.111	-0.029	0.209	0.100	-0.038	0.136	-0.096	-0.186	0.044	0.119	0.045	1	-0.115	-0.221	0.000	0.311
Bettle	0.227	0.424	0.277	0.192	-0.201	0.021	0.073	-0.150	0.131	-0.080	-0.047	0.090	-0.115	1	0.275	0.076	0.155
Ant	0.049	0.157	0.070	0.208	-0.140	-0.074	0.094	0.010	0.215	0.112	0.008	0.001	-0.221	0.275	1	-0.133	0.038
Termite	0.269	0.142	0.193	0.110	-0.051	-0.197	0.074	-0.245	-0.046	-0.145	0.001	0.015	0.000	0.076	-0.133	1	-0.074
Snails	0.291	0.049	0.078	0.089	0.023	0.017	-0.074	-0.056	-0.112	-0.025	0.007	-0.118	0.311	0.155	0.038	-0.074	1

Supplementary Table S11. Correlation between mulch macrofauna species with soil chemical indicators, according to the Pearson test.

Values in bold are different from 0 with a significance level $\alpha = 0.05$

Variables	pH	OM	N	Osen_P	Al3+	Mn2+	Earthworms	Millipede	Centipede	Spider	Insect_larve	Springtails	Enchytraeids	Bettle	Ant	Termite	Snails
pH	1	0.103	0.243	0.342	-0.144	0.024	0.164	0.126	0.211	0.098	0.176	0.142	0.333	-0.121	0.144	-0.136	0.071
OM	0.103	1	0.507	0.039	-0.145	0.018	0.005	-0.131	-0.050	0.095	-0.025	-0.110	0.125	-0.152	0.059	-0.160	0.108
N	0.243	0.507	1	0.189	-0.064	0.146	-0.007	-0.059	0.038	0.171	0.025	0.134	0.209	0.059	0.042	0.027	-0.026
Osen_P	0.342	0.039	0.189	1	0.207	0.071	0.142	-0.236	0.221	-0.053	0.036	0.072	0.301	0.027	0.110	0.255	0.203
Al3+	-0.144	-0.145	-0.064	0.207	1	0.073	0.114	-0.060	-0.103	0.011	-0.070	-0.112	-0.113	-0.091	-0.156	-0.055	-0.042
Mn2+	0.024	0.018	0.146	0.071	0.073	1	0.275	0.050	0.044	-0.110	-0.111	-0.061	-0.150	-0.078	-0.202	-0.023	-0.199
Earthworms	0.164	0.005	-0.007	0.142	0.114	0.275	1	-0.286	-0.152	0.141	-0.117	0.011	-0.030	-0.146	0.070	-0.141	-0.055
Millipede	0.126	-0.131	-0.059	-0.236	-0.060	0.050	-0.286	1	0.287	-0.201	0.142	0.023	-0.021	-0.104	-0.125	-0.109	0.170
Centipede	0.211	-0.050	0.038	0.221	-0.103	0.044	-0.152	0.287	1	-0.104	0.051	-0.025	0.281	-0.041	-0.045	-0.110	0.078
Spider	0.098	0.095	0.171	-0.053	0.011	-0.110	0.141	-0.201	-0.104	1	0.238	0.144	-0.162	0.099	-0.046	-0.050	-0.026
Insect_larve	0.176	-0.025	0.025	0.036	-0.070	-0.111	-0.117	0.142	0.051	0.238	1	0.115	-0.119	0.012	0.178	-0.099	0.199
Springtails	0.142	-0.110	0.134	0.072	-0.112	-0.061	0.011	0.023	-0.025	0.144	0.115	1	-0.080	0.281	0.078	0.093	0.077
Enchytraeids	0.333	0.125	0.209	0.301	-0.113	-0.150	-0.030	-0.021	0.281	-0.162	-0.119	-0.080	1	-0.139	0.097	-0.053	-0.068
Bettle	-0.121	-0.152	0.059	0.027	-0.091	-0.078	-0.146	-0.104	-0.041	0.099	0.012	0.281	-0.139	1	-0.060	0.510	-0.022
Ant	0.144	0.059	0.042	0.110	-0.156	-0.202	0.070	-0.125	-0.045	-0.046	0.178	0.078	0.097	-0.060	1	-0.068	0.087
Termite	-0.136	-0.160	0.027	0.255	-0.055	-0.023	-0.141	-0.109	-0.110	-0.050	-0.099	0.093	-0.053	0.510	-0.068	1	-0.045
Snails	0.071	0.108	-0.026	0.203	-0.042	-0.199	-0.055	0.170	0.078	-0.026	0.199	0.077	-0.068	-0.022	0.087	-0.045	1

Supplementary Table S12. Permutational analysis of variance (PERMANOVA) analysis results for the effect of lime amendment and soil type on soil bacterial, fungal and AMF community composition. Values in bold are significantly different at $P < 0.05$.

Treatment	Soil bacterial community		Soil fungal community		Soil AMF community	
	R^2	P	R^2	P	R^2	P
Liming	0.013	0.174	0.01	0.273	0.03	0.69
Land type	0.04	<0.001	0.04	<0.001	0.07	<0.001
Liming x land type	0.07	<0.001	0.09	<0.001	0.99	<0.001

Appendix 7

Supplementary Table S13. Two-way analyses of variance (ANOVA) results for the impact of liming, soil use history and their interaction on diversity indexes of soil bacterial and fungal diversity indicators. Results in boldface are statistically different ($P < 0.05$).

Source/Treatment	Soil bacterial communities								Soil fungal communities							
	Evenness		Richness		Shannon		Simpson		Evenness		Richness		Shannon		Simpson	
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>
Liming	0.58	0.445	0.05	0.814	0.10	0.74	0.01	0.896	5.313	0.99	6.73	0.60	2.644	0.10	0.983	0.324
	9		5		4	8	7			4	7	8		7		
Land type	0.21	0.643	0.25	0.018	0.39	0.02	0.03	0.86	<0.0001	0.02	0.06	0.01	0.13	0.09	0.082	0.015
	7		0			4	1	1		3	5	1				
Liming x land type	0.01	0.903	0.28	0.896	0.04	0.81	0.28	0.596	0.041	0.84	0.04	0.84	0.008	0.93	0.075	0.785
	5		4		5	5	3			0	1	0		0		

Supplementary Table S14. Summary of soil and root AMF biological indicators with different treatments (mean \pm SD). In each indicator, values in bold or followed by different letters are significantly different at $P < 0.05$, according to the two-way ANOVA and Tukey (HSD) test.

Treatment	Soil AMF communities								Root AMF colonization		
	Evenness		Richness		Shannon		Simpson		Treatments	F (%)	M (%)
	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	<i>F</i>	<i>P</i>	Original control	93.3 \pm 5.17b	32.2 \pm 4.34c
Liming	0.24	0.62	1.96	0.16	0.43	0.51	0.66	0.41	Original lime	97.5 \pm 3.73a	36.4 \pm 3.86b
Land type	14.90	<0.001	12.9	0.001	17.6	<0.0001	12.7	<0.001	Converted control	92.4 \pm 8.17a	33.9 \pm 4.47ab
Liming x land type	0.88	0.35	0.45	0.50	0.64	0.42	0.8	0.37	Converted lime	96.51 \pm 4.89ab	40.2 \pm 4.55a

Table S15. Correlation between soil indicators, mulch and soil macrofauna abundance, soil microbial community abundance and root AMF colonization, according to the Pearson test. Values in bold are different from 0 with a significance level alpha = 0.05

Variables	pH	OM	N	Olsen_P	Al3+	Mn2+	Soil macrofauna abundance	Mulch macrofauna abundance	Bacterial abundance	Fungal abundance	AMF abundance	AMF frequency	AMF intensity	Tea yield
pH	1	0.075	0.168	0.288	-0.057	0.071	0.482	0.398	0.142	-0.008	-0.397	0.118	0.369	0.507
OM	0.075	1	0.453	0.026	-0.100	0.108	-0.038	-0.095	0.123	0.009	-0.126	-0.048	0.015	0.109
N	0.168	0.453	1	0.168	-0.061	0.104	0.158	0.065	0.344	0.110	-0.306	-0.037	0.113	0.225
Olsen_P	0.288	0.026	0.168	1	0.207	0.155	0.279	0.157	0.051	-0.016	-0.194	-0.053	-0.014	0.229
Al3+	-0.057	-0.100	-0.061	0.207	1	0.146	-0.030	-0.164	-0.205	0.193	0.063	-0.274	-0.488	-0.166
Mn2+	0.071	0.108	0.104	0.155	0.146	1	0.019	0.039	-0.232	0.029	-0.100	-0.099	-0.207	0.065
Soil macrofauna abundance	0.482	-0.038	0.158	0.279	-0.030	0.019	1	0.477	0.007	0.062	-0.279	0.116	0.222	0.234
Mulch macrofauna abundance	0.398	-0.095	0.065	0.157	-0.164	0.039	0.477	1	0.021	0.014	-0.200	0.296	0.423	0.295
Bacterial abundance	0.142	0.123	0.344	0.051	-0.205	-0.232	0.007	0.021	1	-0.009	-0.308	0.120	0.387	0.333
Fungal abundance	-0.008	0.009	0.110	-0.016	0.193	0.029	0.062	0.014	-0.009	1	0.155	0.099	-0.051	0.034
AMF abundance	-0.397	-0.126	-0.306	-0.194	0.063	-0.100	-0.279	-0.200	-0.308	0.155	1	0.070	-0.230	-0.519
AMF frequency	0.118	-0.048	-0.037	-0.053	-0.274	-0.099	0.116	0.296	0.120	0.099	0.070	1	0.589	0.275
AMF intensity	0.369	0.015	0.113	-0.014	-0.488	-0.207	0.222	0.423	0.387	-0.051	-0.230	0.589	1	0.407
Tea yield	0.507	0.109	0.225	0.229	-0.166	0.065	0.234	0.295	0.333	0.034	-0.519	0.275	0.407	1

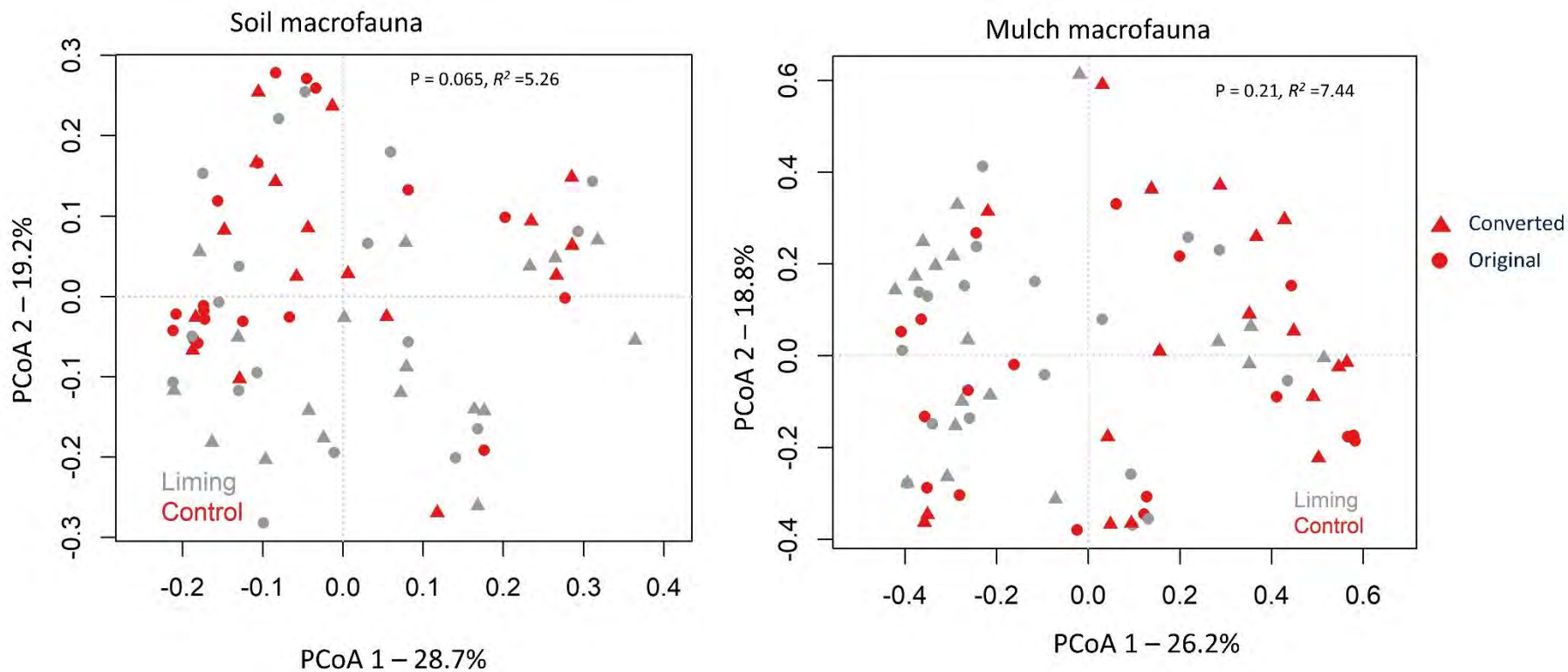


Fig. S1. Principal coordinate analysis (PCoA) representing soil and mulch macrofauna community composition according to liming effect. Explained variations (R) and significance (P value) of each factor from the Permutational multivariate ANOVA over the community dissimilarity matrixes are presented.