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REGULAR PAPER

The first report of composition and occurrence of myxomycete assemblages in protected and unprotected plantation forests: a comparative study in Thai Nguyen City, Northern Vietnam

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Background and aims – In spite of the cosmopolitan distributions of myxomycetes, relatively few studies have been conducted in subtropical forests of Southeast Asia, particularly in Northern Vietnam, where comprehensive reports of myxomycetes are lacking up to this date. Hence, a rapid biodiversity assessment was conducted in protected and unprotected plantation forests of Thai Nguyen City to compare the species composition of myxomycete communities between the two different forest management types.
Methods – A total of 360 substrate samples were haphazardly collected within 5 m × 5 m plots established within three protected and three unprotected forests. The moist chamber cultures prepared from these samples were regularly checked for myxomycete fructifications over a period of 12 weeks. Analyses of diversity (species richness) and community composition were performed using the software EstimateS and the vegan package in R.
Key results – The study yielded a total of 505 records representing 54 species belonging to 17 genera. All species recorded herein were new records for Northern Vietnam; among them five were new for the entire country. The rarefaction curves showed higher numbers of myxomycete species to be expected for protected forest (43.0) in comparison to unprotected forests (39.4). However, calculations of species diversity indices showed higher values in unprotected forest than in protected forest. The species composition between the two forest types showed clear overlaps with many shared (56%) species.
Conclusions – Forest conservation strategies do not affect freely dispersing myxomycetes implicating that spore dispersal of myxomycetes is caused not only by natural factors but also by anthropogenic activities. The production of high number of myxomycete species for this study seems to point out that the subtropical forests of Vietnam harbours many undiscovered myxomycete species for Southeast Asia.

Key words – α diversity, β diversity, Amoebozoa, forest management, morphospecies, plasmodial slime moulds.

INTRODUCTION

With the unprecedented and increasing damage done in many ecosystems around the world, billions of dollars have been invested globally (Turner & Daily 2008) in habitat protection as a strategy to conserve plants, animals, and ecosystems (Chen et al. 2017) in the face of threats such as land use change, habitat fragmentation, and invasive species. Protecting an area is considered to be an essential strategy for maintaining diverse biological components, as has been shown by some studies that intends to look on the species diversity between protected and unprotected areas (Sedberry et al. 1999, Devkota et al. 2010, Dhaou et al. 2010, Orimaye et al. 2016, Burgess et al. 2017). However, despite these substantial studies about species diversity in association with the area of protection, most of the attention of these studies was focused on macroscopic organisms. Only a few studies were dedicated to investigate the effect of conservation on species composition of microorganisms. Myxomycetes, living as true micro-organisms (Schnittler et al. 2012) but visible by their fructifications which have been studied for nearly 200 years (Stephenson et al. 2008), are an ideal group to investi-

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gate the effectiveness of conservation measures for microorganisms. Assessing microbial diversity is important because of their vast numbers, and many groups are vital players in keeping ecosystems functioning.

Myxomycetes, also known as myxogastrids or plasmodial slime molds, are phagotrophic eukaryotic microorganisms which occur nearly in all types of terrestrial ecosystems. While most morphospecies (further designated as 'species') are cosmopolitan in distribution some are restricted to the tropics or subtropics (Schnittler et al. 2017) or are reported only in temperate regions (Alexopoulos 1963, Farr 1976, Martin et al. 1983). For the past three decades, a number of myxomycete diversity studies have been conducted in different regions of the world. Still, most of the studies are conducted in temperate regions (Eliasson 1981, Ing 1983, Stephenson 2003, Novozhilov et al. 2006, Lado et al. 2007), although the number of surveys carried out in the tropics or subtropics, especially in the Southeast Asian region, is steadily growing. Even so, only two countries in the latter region have been intensely surveyed in comparison to the other neighbouring countries that published merely species lists. These are the Philippines and Thailand having 158 (Dagamac & dela Cruz 2015, Macabago et al. 2017) and 132 (Ko Ko et al. 2010) annotated myxomycetes species, respectively. In comparison, only a limited number of diversity and ecological studies have been completed in Vietnam, a region known to have a rich and diverse vegetation, and contrasting seasonality.

The first myxomycete report in the country came from van Hooff (2009) who provided an initial annotated list of 23 species, with *Cribraria tecta* Hooff described as new for science. Additionally, two new species, *Diderma cattiense* Novozh. & D.W.Mitchell and *Diderma pseudotestaceum* Novozh. & D.W.Mitchell, were reported by Novozhilov et al. (2014). An ecological study focused on three lowland tropical forests in Vietnam (Tran et al. 2014) and added 32 new records of myxomycetes species. Assessment of myxomycetes occurring on common agricultural leaf litters in Vietnam such as *Camelia sinensis* (L.) Kuntze, *Dimocarpus longan* Lour. and *Psidium guajava* L. have also been recently reported (Redeña-Santos et al. 2017). The most comprehensive study ever conducted for the country was the biodiversity assessment conducted in Southern Vietnam by Novozhilov et al. (2017), adding a total of 69 species recorded as new for Vietnam. Altogether, there are 126 myxomycete species known for the country (Tran et al. 2017). Looking at regions, Southern Vietnam is much better studied than Northern Vietnam. Therefore, the primary objective of this study was to obtain the baseline data for Northern Vietnam, with the second goal to investigate value of these organisms for monitoring effectiveness of conservation measures for maintaining microbial diversity using the plantation forests in Thai Nguyen City. To ensure comparability within habitats, the moist chamber culture technique was employed, were myxomycete fructifications can be induced in a controlled environment (Härkönen 1979, 1981).

MATERIALS AND METHODS

Collecting localities

Thai Nguyen City (21°35′39.19″N, 105°50′53.41″E) has a total land area of approximately 189 705 km^2. The climate in the city is generally characterized to be humid subtropical climate with relatively high temperatures and evenly distributed precipitations. Within the vicinity of Thai Nguyen City (fig. 1A), two community forests were classified as protected and unprotected forests. Both forest types are characterized as plantation forest mainly dominated by the non-indigenous plant species *Acacia mangium* Willd. with natural regeneration of native species under the forest canopy. The collecting localities for the protected forest (fig. 1B) were within the well-managed Núi Cốc Lake which serves as an ecotourism park for the city, and have, in major parts, a closed canopy cover. Since majority of the localities in the protected plantation forests are managed, it is only exposed to moderate human activities usually in the purpose of leisure or nature sightseeing. On the other hand, the collecting localities for the unprotected forests (fig. 1C) have more open canopies, and are freely accessible to human-mediated disturbances, i.e. grazing of livestock, timber logging and since they are usually found along the road, they are highly exposed to many vehicular transports.

Collection of substrates, laboratory isolation method and myxomycetes characterization

For both the protected and unprotected plantation forest, three collecting localities were randomly chosen based on their accessibility. Three 5 m × 5 m plots were then initially established for each of the three collecting localities. Five samples from four types of substrates (aerial leaf litter – AL, ground leaf litter – GL, dead twigs – TW and bark of living trees – BK) were collected arbitrarily within each plot, resulting in a total of 60 samples per collecting locality, 180 samples per forest type and 360 samples for the whole study. All samples were placed inside dry paper bags and were transferred immediately in the culture laboratory of the Institute of Botany and Landscape Ecology, University of Greifswald to set up the moist chamber cultures (MC) following the protocol of Stephenson & Stempen (1994). AL and GL substrates were cut to c. 1 cm × 1 cm size; TW and BK substrates were broken into 10–15 pieces and 5–7 pieces of roughly this size and placed in a way that nearly the entire area of a Petri dish of 9 cm diam. was covered. Cultures were soaked overnight in sterile distilled water. After 24 hours, the pH of each substrate was checked with an Orion model 610 pH-meter while the excess water was removed up to the point adequate enough for the chamber to be moist. The moist chamber setups were maintained under ambient lighting conditions and placed inside wooden cabinets not directly exposed to sunlight, incubated at room temperature (22–25°C) for up to 12 weeks and were checked at least every week for the presence of myxomycete plasmodia and/or fructifications. If the moist chamber dried out for the first time and no plasmodia and fructifications were observed, water was added to keep the culture moist.

For MCs that yielded fructifications, substrate was initially glued in herbarium boxes and was cooled inside a refrigerator overnight. For the initial myxomycetes determination, the specimens were investigated based on their macroscopic characters using the structures of their fructification under a stereo microscope (Zeiss Stemi DV4). In addition, microscopic characters using the features of the spores and capillitium were examined by putting a single fruiting body on a slide with a small drop of Hoyer's medium. After 24 hours, when the spores became more distinctly rounded in shape, the aforementioned microscopic characters were observed under a compound light microscope (Zeiss Axiolab A1). The specimens were then determined using published literature (Martin & Alexopoulos 1969, Poulain et al. 2011) and with a web-based identification key (http://slimemold.uark.edu/). Nomenclature follows Lado (2005–2017). The abbreviation 'cf.' was put next to the taxon name for specimens that could not be distinguished with certainty due to some malformations but still showed enough adequate characteristics to be identified as a species. All specimens recorded herein were deposited in the herbarium of the Institute of Botany and Landscape Ecology, Greifswald University (GFW).

Data evaluation

Initially, the productivity of MCs was recorded for each forest type. Here, a moist chamber that exhibited either plasmodial and/or fruiting body growth was considered as positive MC for myxomycetes. Thus, was noted as one positive record. The number of positive MC was counted and divided by the total number of MCs prepared. Species composition and occurrence was also determined. The relative abundance of each species was obtained by dividing the total number of records for each species of myxomycetes by the total number of myxomycetes collected (Stephenson et al. 1993). An abundance index was then translated from the computed values as described by Stephenson et al. (1993), wherein < 0.5% – rare (R); > 0.5% but < 1.5% – occasional (O); > 1.5% but < 3% – common (C); > 3% – abundant (A). The taxonomic diversity (S/G ratio) was then computed by getting the quotient of the number of species (S) with the number of genera (G). The lower the S/G ratio, the more diverse a particular biota is considered since in principle a biota in which the species were divided among numerous genera is intuitively more diverse in a taxonomic sense than a biota wherein the species belong only to a few genera (Stephenson et al. 1993).

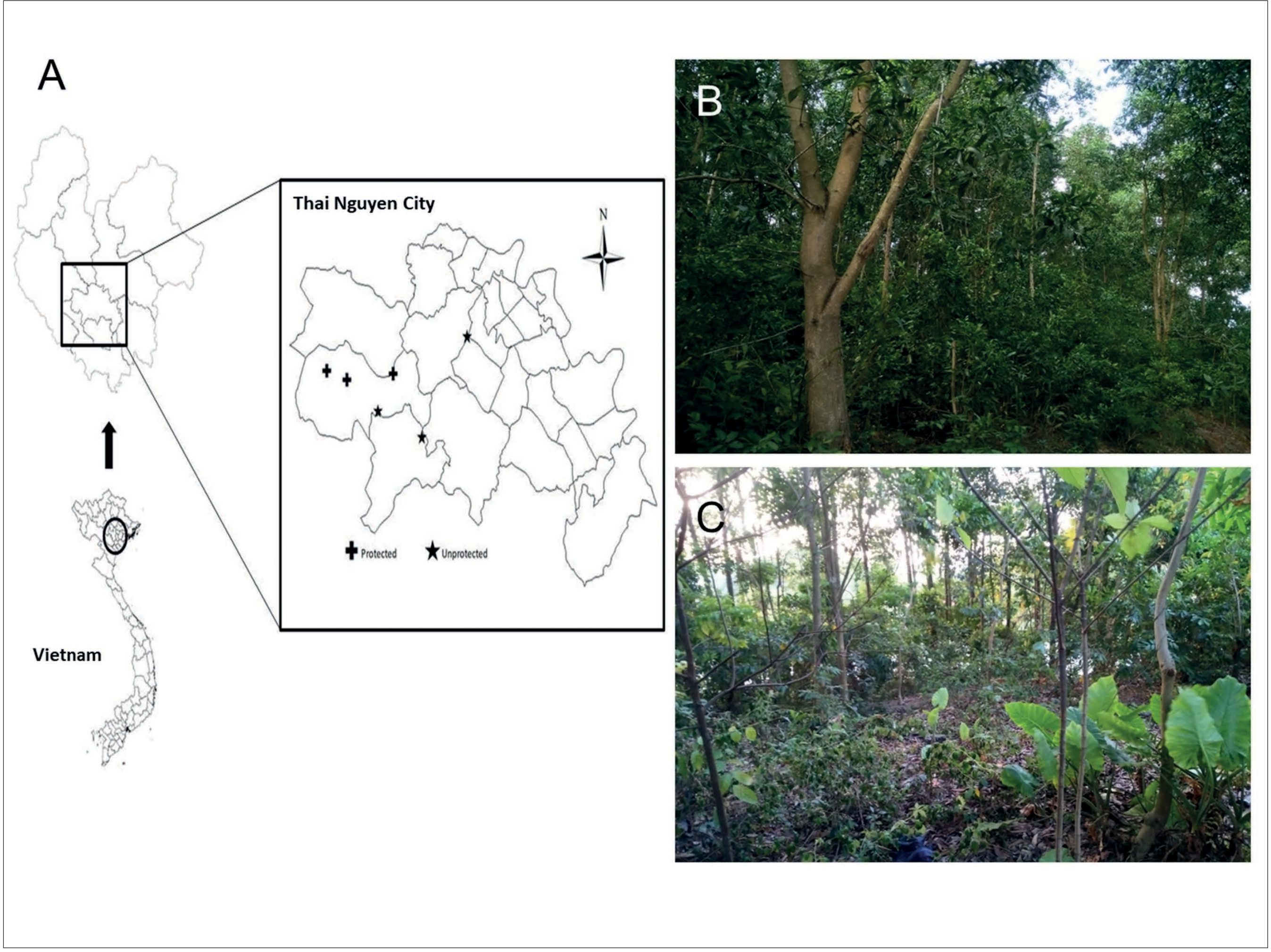

Figure 1 – A, map of Thai Nguyen City showing the three points where substrates were collected in protected (cross) and unprotected (star) plantation forests and image of the collecting localities for: B, protected and C, unprotected plantation forests.

To estimate the survey exhaustiveness, the software program EstimateS (Version 9.1, Colwell 2014, 100 randomizations) was used to construct a species accumulation curve (SAC). The Chao 1 estimator results (an estimator that targets richness for individual based data as such that one record of a species in a certain culture is considered as one individual) was then utilized in accordance with the protocols of Macabago et al. (2017) and Novozhilov et al. (2017). The estimated value for the percentage of completeness for each study sites was then calculated by dividing the actual number of species recorded by the mean number of species expected as estimated by the Chao 1 estimator. Rarefaction curves, a method to reduce samples of different sizes to a standard size, as to make them comparable in terms of number of species, for each study sites were then constructed from the Coleman rarefaction values generated from EstimateS to compare the species richness between the two types of plantation forests.

Using the vegan package in the R environment and the R scripts from Dagamac et al. (2017a), the α diversity, expressed as Fisher (FIS) Indices for species richness, Shannon (SHA) and Simpson (SIM) that measures heterogeneity, and the three Hill series (N0 = species richness only, N1 = exponent of the Shannon diversity, N2 = inverse of the Simpson diversity) were generated. Subsequently, a boxplot was produced from the six diversity measurements to illustrate the diversity between the two forest types. For statistical significance of the indices, t-tests using PAST software (Hammer et al. 2001) were performed. Also, the β diversity or community analysis between the two forest types was assessed using an ordination analysis employing the non-metric multidimensional scaling (NMDS) based on the Bray Curtis similarity distances using the metaMDS function in R studio. The statistical test Permutation Analysis of Variance (PERMANOVA) based on 999 permutations was performed using the *adonis* function of R.

RESULTS

The present study provided 505 total records (including 420 from fruit bodies and 85 from plasmodia). All of which were new records for Thai Nguyen City, and 5 are reported herein as new records for the country. For the entire study 87% of all MCs were positive for myxomycetes; figures for protected and unprotected forests were 80% and 94%, respectively.

Using EstimateS, the rarefaction curve (fig. 2A) indicated a higher number of species for protected forest (43.0) than for unprotected forest (39.4). Furthermore, the computed values of Chao 1 estimators clearly displayed a well-sampled survey for both protected forests with 88% (fig. 2B) and 92%

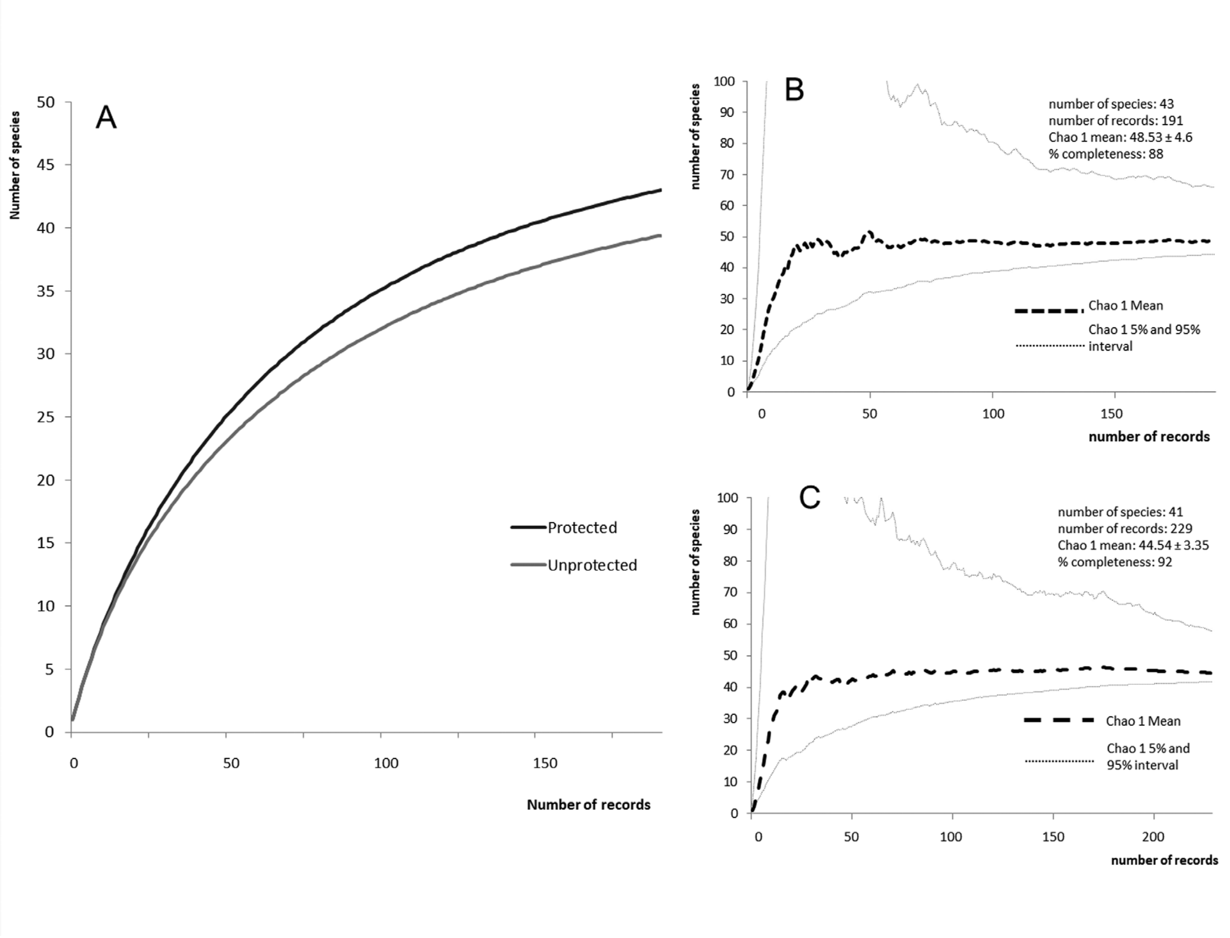

Figure 2 – A, rarefaction curves for two different plantation forest types and the generated sample based species accumulation curves from Chao 1 mean estimator for: B, protected and C, unprotected forests.

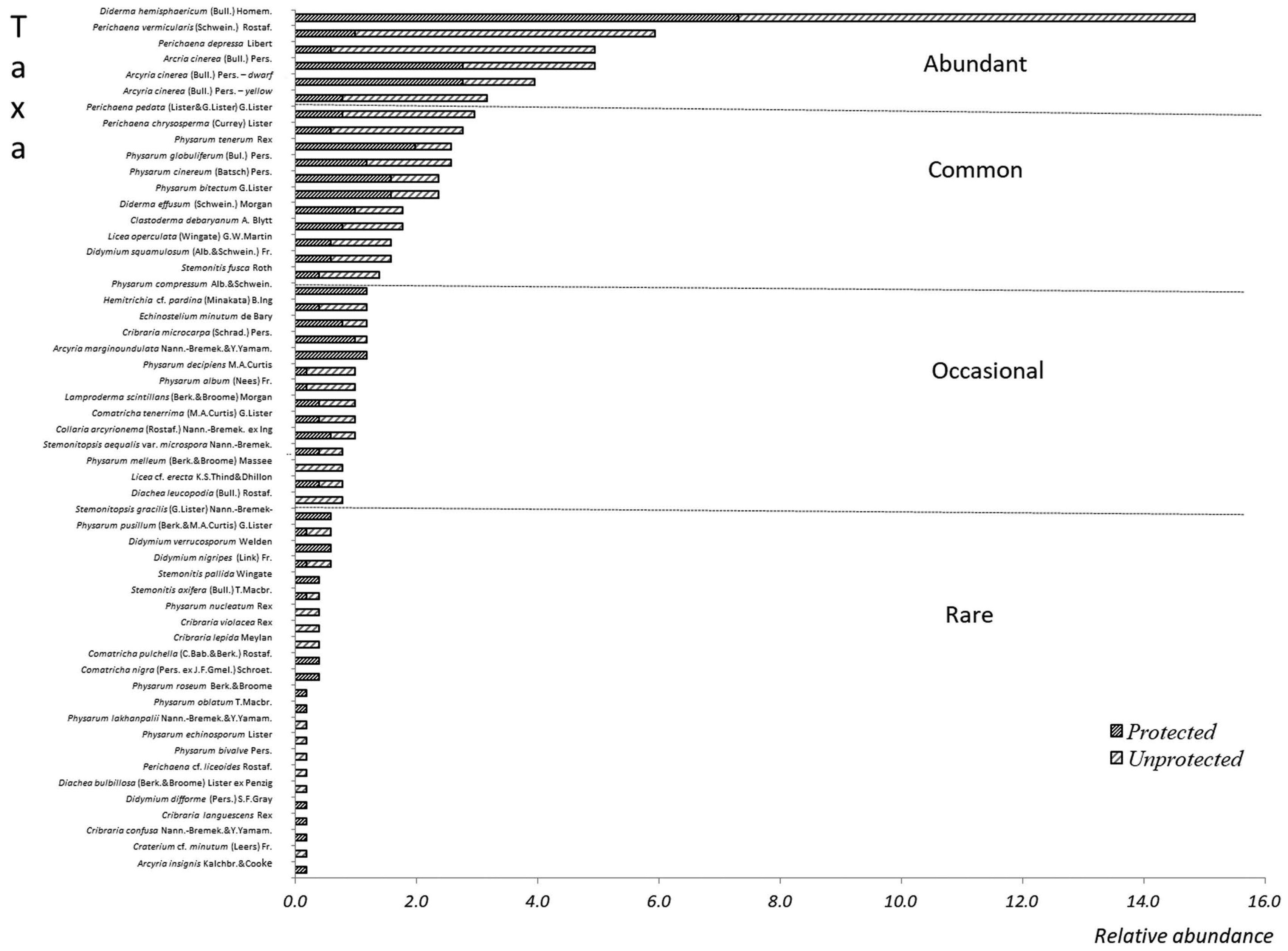

Figure 3 – Myxomycete occurrence and their respective relative abundances between different forest types.

(fig. 2C) completeness, respectively. When determining the relative abundance (fig. 3), 23 species were reported to be rare, 14 were recorded as occasionally occurring, while 11 were common. In addition, six species were abundant for the entire study and these are *Arcyria cinerea*, *A. cinerea* (dwarf form, see Schnittler 2000), *A. cinerea* (yellow form), *Diderma hemisphaericum*, *Perichaena depressa* and *P. vermicularis*.

In terms of taxonomic diversity, the 54 species reported in the entire study belonged to 17 genera. Between the two types of forest, unprotected forest was found to be taxonomically more diverse (S/G = 2.41) than the protected forest (S/G = 2.87). This was evident from the 43 species representing 15 genera in the protected forest, whereas 41 species with 17 genera were recorded for the unprotected forest. Although there were more different species in protected forest, more genera were represented in unprotected forest. The α diversity between different plantation forest type was also computed using six different diversity indices namely Fisher's alpha, Shannon's index, Simpson's index and three Hill diversity series. According to the generated boxplot (fig. 4), Shannon's index, Simpson's index, Hill's N0, Hill's N1 and Hill's N2 showed a clear trend as they gave a higher value for unprotected than the protected forests, indicating that unprotected forest had a more diverse myxomycete assemblage than protected forest. However, the difference was not statistically significant ($p > 0.05$, t-test). Meanwhile, as for β diversity, the NMDS ordination (fig. 5) exhibited no significant differences in species composition between protected forest and unprotected forest ($R = 0.163$, $p > 0.05$) as the two dispersion ellipses overlapped with each other indicating that the communities of the two forest types shared a high number of similar species.

DISCUSSION

Most of what is known about the ecology of myxomycetes has been a result from studies mainly conducted to assess myxomycetes assemblages in association with different terrestrial ecosystems (Beltrán-Tejera et al. 2010, Rollins & Stephenson 2013, Dagamac et al. 2015a, 2017b, Macabago et al. 2016, Redeña-Santos et al. 2017), different microhabitats (Schnittler & Stephenson 2002, Ndiritu et al. 2009, Coelho & Stephenson 2012, Carascal et al. 2017), and different environmental factors such as surface fire (Adamonytė et al. 2016), disturbance (Dagamac et al. 2015b) and elevation (Rojas & Stephenson 2008, Dagamac et al. 2014, Rojas et al. 2016). However, ecological studies that take into account protection of plantation forests is still relatively scarce. Thus, this rapid assessment of myxomycete biodiversity using the moist chamber technique in Thai Nguyen City wanted to investigate if protection measures against exposure to human-mediated disturbance will have differences in terms of myxomycetes diversity and community structure.

Our results showed that of the 505 total records, 420 (83%) determinable fructifications were noted while 85 (17%) were plasmodial records. This is evident from the high moist chamber (MC) productivity for the entire study (87%). Judged according to the generated species accumulation curve (SAC), the sampling survey for both forest types seems to be sufficient to recover all of the most possible common species in the area. This could be attributed to the efficiency of the MC technique to supplement the field col-

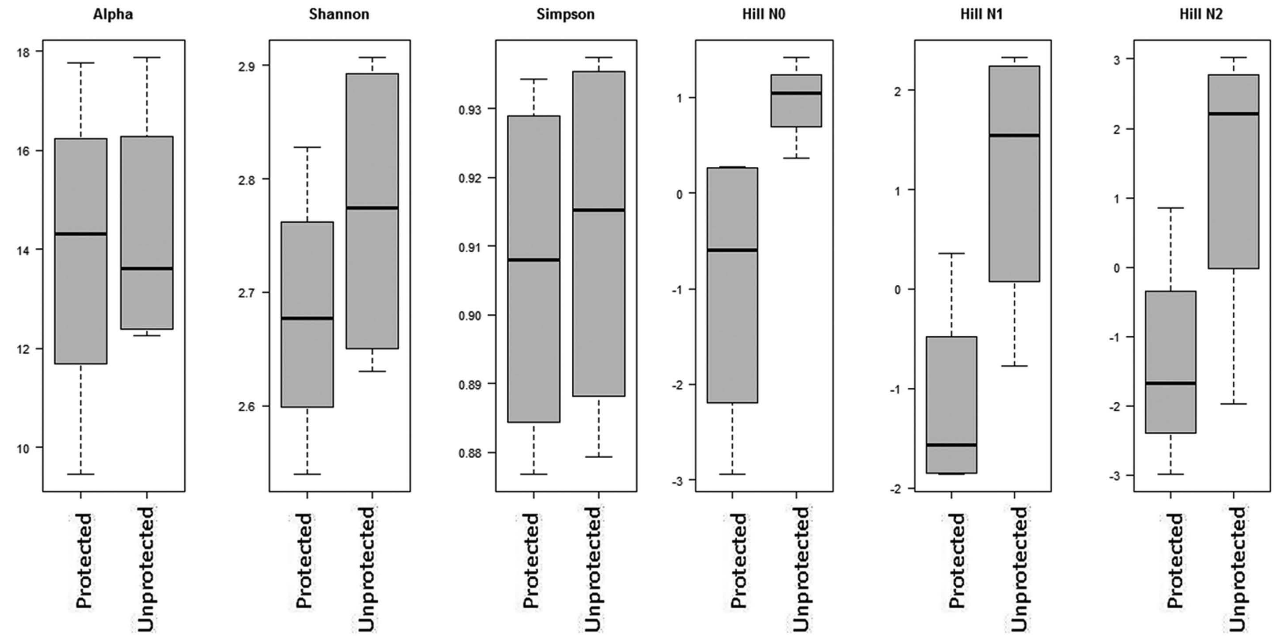

Figure 4 – Box plot showing the comparison of six different diversity indices (Alpha = Fisher's alpha; Shannon = Shannon's *H* index; Simpson = Simpson's diversity index; N0 = species richness only, N1 = exponent of the Shannon diversity, N2 = inverse of the Simpson diversity).

lections of fructifications, especially on areas with extreme environments where fructification development is a challenge. However, many species, especially those with large compound fructifications, are rarely fruiting in MCs, therefore this method alone cannot adequately reflect the entire myxomycete diversity of an area (Novozhilov et al. 2017). But especially in the tropics where fructifications can decay fast, the MC technique is also an effective set up to assess the diversity of myxomycetes (Corpuz et al. 2012, Dagamac et al. 2012, Tran et al. 2014, Pecundo et al. 2017). The percentage yield on this paper (87%) is comparatively higher than the 51% productivity recorded in protected ecoparks in the Philippines (Macabago et al. 2010). Perhaps, the difference in a subtropical climate could be a factor for this percentage yield. Nevertheless, in spite of solely using the moist chamber technique due to financial and time constraints, this study showed the efficiency of MC technique in conducting rapid biodiversity surveys for myxomycetes. However, for further investigation of myxomycetes assemblages, field surveys are recommended to ensure comparability with other studies.

The rarefaction curves indicated that the protected forest has a higher expected number of species than the unprotected forest. This result was further supported by the Fisher's alpha index which measures species richness only. However, in terms of taxonomic and species diversity, the results were reversed. In both cases the unprotected forest gave a higher value than the protected forest. This contrasts with other studies, were it has been observed that protected areas often recorded higher species diversity than unprotected areas (Dhaou et al. 2010, Gray et al. 2016, Orimaye et al. 2016). This is based on the supposition of biodiversity conservation for macroorganisms that states that the essence of habitat protection intends to maintain diverse biological components. Meanwhile, microorganisms that are of smaller in size are said to confound on the ubiquitous model which state that "everything is everywhere, but the environment selects". Thus, it seems that freely dispersing microorganisms such as myxomycetes are not directly affected by conservation strategies but rather by the intensity of disturbances (Rojas & Stephenson 2013, Macabago et al. 2017). This may be the case for the unmanaged unprotected forests which suffer from anthropogenic activities i.e. logging, slash and burn farming, or hunting, that destruct the forest but add to habitat heterogeneity, resulting in higher species diversity and richness ar-

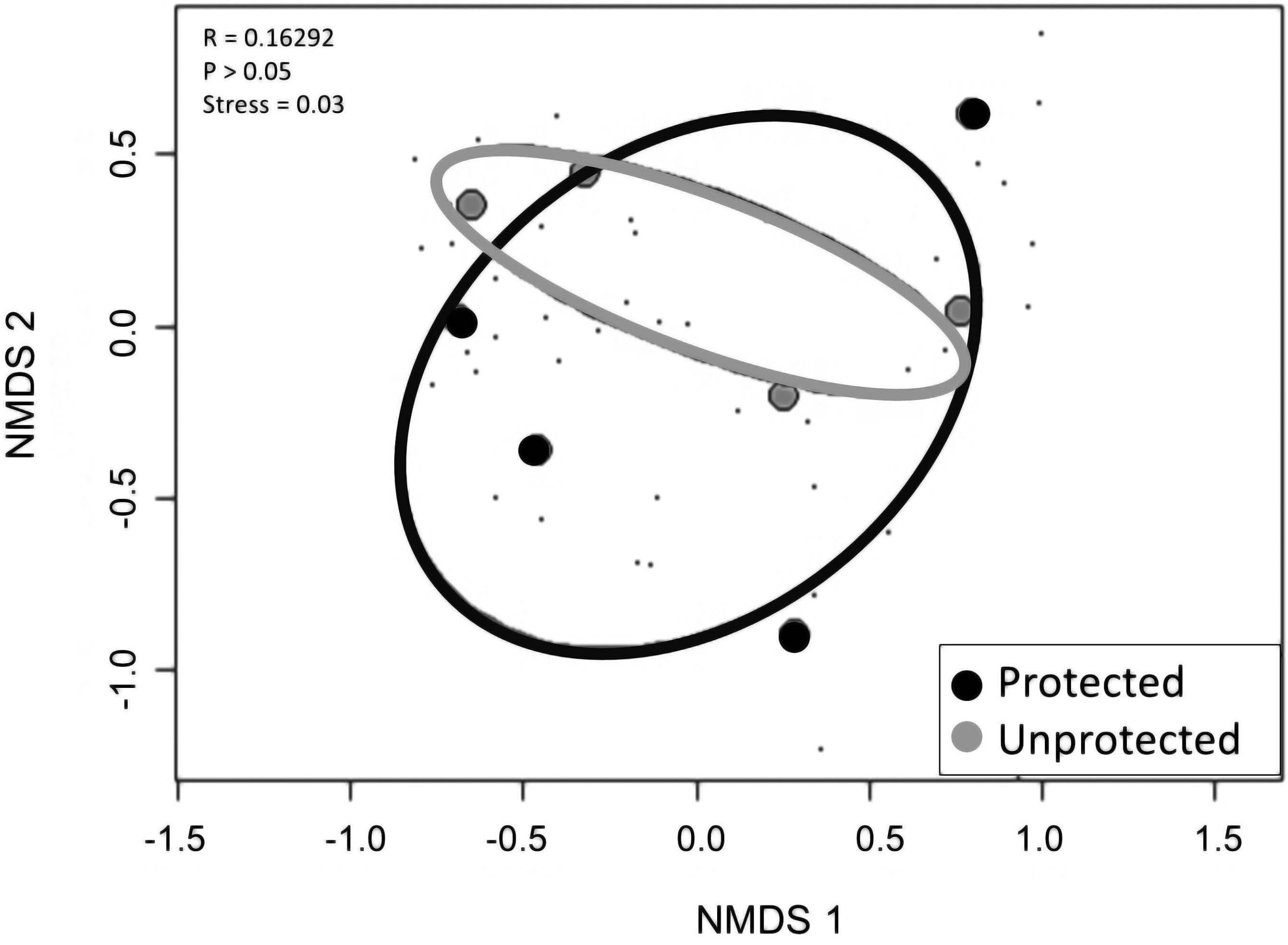

Figure 5 – Non-metric multidimensional scaling (NMDS) of species occurrence between two forest types. Black dots represent the position of myxomycetes species in the ordination space. Black and grey circles represent the forest type; ellipses indicate dispersion based on standard deviation of point scores.

eas. In other related studies on the level of management of different forest types, unmanaged types of forests are known to cover more species than managed forests (Økland et al. 2003). A meta-analysis of Paillet et al. (2009) evaluating 49 published papers containing 120 individual comparisons of species richness between managed and unmanaged forest throughout Europe concluded that species richness tends to be higher in unmanaged forests for taxonomic groups like saproxylic beetles, bryophytes, lichens and fungi. All these taxa are substrate-dependent taxa and may easily suffer from a reduction of microhabitat availability and diversity in managed protected forest. Thus, myxomycetes may also follow this trend: on one hand higher diversity and availability of plant-derived substrates, like in disturbed, unprotected areas, may result in higher myxomycete diversity. On the other hand, forest protection increases canopy cover, decreases disturbance and thus plant and substrate diversity, which decreases myxomycete diversity. But, simultaneously substrate quality may increase (i.e. by providing coarser woody debris). These two trends may well cancel each other out.

In terms of species turnover, a clear overlapping of myxomycete assemblages was observed in the NMDS ordination. The statistical test PERMANOVA also accepts the null hypothesis that the species composition of protected forest has no significant difference on the unprotected forest ($p > 0.05$). This is highly possible since myxomycetes are known to have the capability of long-distance dispersal (Kamono et al. 2009) in some instances, where airborne spores in the area would have the potential of being dispersed by wind over considerable distances. A study by Schnittler et al. (2006) on canopy myxomycetes concluded that spores can easily be dispersed up to 1.8 km by even a slight breeze and theoretically up to 500 km by a storm with a speed of 100 km/h. However, dispersal is simply a function of distance. For a successful dispersal between isolated habitat patches or areas to occur, it needs direction. Therefore, understanding by which spores move through dispersal vectors is needed. Additionally, it is also more important to understand where and how these spores can be dispersed by different vectors. Similar with plants, a study shows that birds (Viana et al. 2016) and small mammals (Schickmann et al. 2012, Christianini & Oliveira 2010) can disperse seed directionally between habitats in association with their habitat preferences and by the landscape in which they move. In the present study, human activities and human-mediated dispersal (HDM) may best explain the similarities of species composition between two community forests and thus, consider humans as the vectors for spore dispersal of myxomycetes in a local scale. This can be supported by a research of Wichman et al. (2009) in which they studied the mechanism of human-mediated dispersal of seeds over long distances. Wherein, the mechanistic modelling showed that wind, the primary vector, was less important as an agent of dispersal less than 250 m while walking humans can disperse seeds to very long distances, up to 10 km at least. These could be the same with myxomycete since spores could easily attach to human clothing, footwear, vehicles, etc. in the landscape, transporting available species. Once attached, the mobility of humans can disperse the spores at landscape scales, where humans can easily move species between different sites especially in areas that are highly accessible. However, these speculations are not yet confirmed and thus, the role of humans as the vectors of spore dispersal for myxomycetes needs further investigation.

In comparison with other Southeast Asian countries, although considered as a biodiversity hotspot, the number of taxonomic and ecological studies of myxomycetes in Vietnam can still be considered to be relatively scarce, with most studies carried out in the southern part of the country (van Hooff 2009, Novozhilov & Mitchell 2014, Novozhilov et al. 2014, 2017, Tran et al. 2014, Novozhilov & Stephenson 2015). This study now serves as the first report of 54 first records of myxomycetes for Thai Nguyen City, a species assemblage that can serve as the baseline information for another intensive survey for Northern Vietnam. Five species were new records for the country, namely *Comatricha nigra*, *Licea* cf. *erecta*, *Perichaena* cf. *liceoides*, *Physarum bitectum* and *Stemonitis pallida*. Altogether, this study updates the number of myxomycete species recorded for Vietnam to 131.

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