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Impact of land use change and agricultural practices on aquatic microbial diversity and functioning in a tropical system

Thi Huong Le

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Impact of land use change and agricultural practices on aquatic microbial diversity and functioning in a tropical system

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Summary

Land use (LU) change towards non-sustainable agricultural practices has resulted in soil degradation, erosion, and the loss of soil microbial diversity and functions. These activities in upland soil can affect aquatic ecosystems through catchment hydrological processes. They may increase runoff and the transfer of eroded nutrients and bacteria from soil into stream water and induce shifts in aquatic community diversity. This problem becomes particularly urgent in developing countries where LU change from shifting cultivation to more permanent agriculture and excess fertilizer application is prevalent. The impact of LU change and agricultural practices on soil microbial communities has been well studied, however, the effects of LU change on in-stream microbial community structure remain poorly understood, particularly in tropical ecosystems. The main question of this work is how LU change and its legacy and agricultural practices affect aquatic bacterial diversity and functioning in a tropical system.

To answer this question, I conducted several experiments at different scales including controlled micro-and mesocosms experiments and *in situ* investigations. The *in situ* investigation was conducted in Houay Pano catchment, Laos, which is representative of tropical catchments with rapid LU change from shifting cultivation to annual crops and teak plantations. Throughout this work, dissolved organic carbon (DOC) and its optical characteristic were measured. High throughput sequencing of 16S rRNA genes was used to measure bacterial diversity. Biolog Ecoplates were used to measure metabolic capacity.

For the controlled mesocosm experiment, I studied the response of the aquatic bacterial community to additions of runoff from soil amended with different organic fertilizers (compost, biochar and vermi-compost) during a 16 days incubation. The results indicated that the addition of runoff caused changes in bacterial community structure and diversity in reservoir water. The decrease of richness and evenness of the bacterial community in treatment mesocosm suggested that there was specialization of species when both allochthonous and autochthonous DOC was present.

For the *in situ* investigations, I tested the effect of LU and LU legacy on stream bacterial community at different sites from upstream and downstream in seasonal variation at base flow when the dispersal is low. The results showed that although stream bacterial composition, structure and functioning varied with the seasonal variation of hydrological conditions, LU change and its legacy was the main determinant driving DOC and its optical characteristics and the bacterial community along the stream. Changes in LU, particularly the transition between teak plantation and crops occurring over the previous four years led to an increase in the biodiversity of the particle attached (PA) bacterial community. The transport of DOC and the bacterial community from upland soil into stream via overland flow was assessed during the flood event. The results indicate that overland flow exports allochthonous DOC and the PA bacteria community and that this fraction (PA) was dominant in stream water during the flood event. The community structure of the PA fraction, both upstream and

downstream, was more similar to overland flow than was the FL fraction. Moreover, DOC was found to play an important role in the co-occurrence network in stream water at base flow and was a significant factor driving community structure at high flow during the flood event. In addition, to test how differences in the characteristics of runoff water in terms of DOC and the PA and FL bacterial community, a rain simulation under different LU (TW, TWO and upland rice (UR)) was conducted. The results found that the percentage of common bacteria between runoff and the corresponding soil under TWO (more than 50%) was higher than that in TW and UR for both PA and FL. This suggests that agricultural the practice of burning of understorey in teak plantations (TWO) removes the layer of surface vegetation to which many taxa attached and may explain the erosion bacteria attached to soil particles during the rain simulation. Moreover, I also observed the highest number of specifics (the taxa that only appeared in runoff from one LU) lost from soil in runoff water from TWO. This suggests that some specific taxa in TWO are more sensitive to erosion under rainfall. More importantly, the loss of bacterial groups from soil into runoff during a rain simulation, and from overland flow into stream water during the flood affect the functional groups of upland soil such as groups relating to soil nitrogen cycle.

To support and confirm the results from the *in situ* investigations, I also conducted a microcosm experiment to assess the response of the stream bacterial community when adding overland flow from soil under different LU (2 years and 8 years fallow (2YF, 8YF), TW and TWO during 96 hours incubation. The results from this experiment also confirmed that the shift of bacterial community structure occurred in different treatments with addition of runoff from different soils. The richness and evenness of the bacterial community in the treated microcosms decreased after the 96 hours incubation. Moreover, DOC concentration was significantly related to bacterial community structure in different LU during the 96 hours incubation. Similar to the mesocosm experiment, this suggested a predominance of specific bacterial community groups when soil bacterial community is added into stream water.

The results of this work show the importance of considering both past and present LU along with hydrological processes when assessing stream microbial diversity and functions. While the experiments in controlled conditions (micro-and mesocosms) allow disentangling the relative importance of direct overland flow and soil community on stream bacterial structure, the *in situ* approach give an integrated view at the basin scale. The results of this thesis emphasize the need to use sustainable LU management practices if we wish to mitigate off-site impacts on downstream aquatic systems.

Résumé

Le changement d'utilisation des terres (UT) vers des pratiques agricoles non durables a entraîné la dégradation et l'érosion des sols, et la perte de sa diversité microbienne et de ses fonctions. Les activités humaines dans les sols en amont peuvent affecter l'écosystème aquatique en aval en modifiant les processus hydrologiques au sein des bassins versants. Le ruissellement et l'érosion des nutriments et des bactéries du sol dans les cours d'eau peuvent augmenter, ce qui risque de modifier la diversité des communautés aquatiques. Ce problème devient particulièrement urgent dans les pays en voie de développement où l'UT passe de la culture itinérante à une agriculture plus permanente et où les applications excessives d'engrais sont courantes. L'impact du changement d'UT et des pratiques agricoles sur la communauté du sol a été relativement bien étudié, mais ses effets sur la structure de la communauté microbienne dans les cours d'eau restent mal connus, en particulier dans les écosystèmes tropicaux. La question principale de ce travail est de savoir comment le changement d'UT présent et passé et les pratiques agricoles associées affectent la diversité bactérienne aquatique et leur fonctionnement dans un écosystème tropical.

Pour répondre à cette question, j'ai mené des expériences à différentes échelles, y compris des expériences contrôlées en micro- et méso-cosmes et des investigations *in situ*. Ces dernières ont été menées dans le bassin versant de Houay Pano, au Laos. Cette zone est représentative d'un bassin versant tropical classique suivant un changement rapide d'UT, passant de la culture itinérante aux cultures annuelles et aux plantations de teck. Tout au long de ce travail, le carbone organique dissous (COD) et ses caractéristiques optiques au sein d'échantillon du cours d'eau ont été mesurés. Le séquençage à haut débit des gènes de l'ARNr 16S a été utilisé pour mesurer la diversité bactérienne au sein des mêmes échantillons. Enfin, les plaques Biolog Ecoplate ont été utilisées pour mesurer la capacité métabolique des communautés aquatiques.

Tout d'abord dans une expérience de mésocosmes contrôlés, j'ai étudié la réponse de la communauté bactérienne aquatique lors de l'ajout d'eaux de ruissellement provenant de différents engrais organiques modifiés dans le sol (compost, biochar et vermicompost) pendant 16 jours d'incubation. Les résultats ont indiqué que l'ajout d'eaux de ruissellements entraînait un changement de la structure de la communauté bactérienne et de la diversité aquatique du réservoir. La diminution de la richesse et de l'équitabilité bactérienne dans les mésocosmes sous traitement a démontré qu'il existait une spécialisation des espèces lorsque le DOC allochtone et autochtone étaient présents. Ensuite, pour l'investigation *in situ*, j'ai testé l'effet de l'UT passée et présente sur la communauté bactérienne du cours d'eau à différents sites en amont et en aval du bassin, et ce à différentes saisons, lorsque le débit de base et la dispersion sont faibles (saison sèche) ou soutenues (saison humide). Les résultats ont montré que la composition bactérienne, la structure et le fonctionnement des cours d'eau variaient avec les variations saisonnières des conditions hydrologiques, mais que l'UT passée était le principal déterminant du DOC,

de ses caractéristiques optiques et de la communauté bactérienne. Les modifications de l'UT, en particulier la transition entre les plantations de teck et les cultures des quatre dernières années, ont entraîné une augmentation de la biodiversité de la communauté bactérienne attachée aux particules (AP).

Le transport du DOC et de la communauté bactérienne du sol en amont vers les cours d'eau via l'écoulement de surface a également été évalué lors d'une crue typique de la saison des pluies. Mes résultats ont indiqué que les écoulements terrestres exportaient des DOC allochtones et une plus grande communauté de bactéries AP qui dominaient les communautés des cours d'eau pendant la crue. La structure de communauté de la fraction AP en amont et en aval du bassin versant était plus semblable à celle de l'écoulement de surface que pour les communautés issues de la fraction libre (FL). Les espèces clé de voûte mises en évidence par des approches en réseau de cooccurrence des communautés échantillonnées pendant la crue appartenaient toutes à la fraction AP et provenaient des écoulements de surface terrestres. De plus, on a constaté que le DOC jouait un rôle important dans le réseau de cooccurrence en saison sèche et qu'il constituait un facteur important de la structure communautaire en période de crue.

En outre, pour tester comment les différences de caractéristiques des eaux de ruissellement liées au DOC, à la communauté bactérienne associées aux fractions AP et FL, une simulation de pluie sous différentes UT (TW, TWO et riz de hautes terres (UR)) a été réalisée. Les résultats ont montré que le pourcentage de bactéries communes entre le ruissellement et le sol correspondant sous TWO (plus de 50%) était plus élevé que dans TW et UR à la fois pour les communautés AP et FL. Cela suggère que les pratiques agricoles visant à brûler le sous-bois sous les plantations de teck (TWO) retirent la couche de végétation de surface où de nombreux taxons vivent. Ces bactéries suivent les particules de sol érodées lors de la simulation de pluie. De plus, j'ai observé un plus grand nombre de taxons spécialistes (ceux n'apparaissant que dans les eaux de ruissellement de TWO) perdus du sol dans les eaux de ruissellement de TWO. Cela suggère que certains taxons spécifiques de TWO sont plus communément retrouvés dans les eaux de ruissellement. Plus important encore, la perte de certains groupes bactériens du sol dans les écoulements sous simulation de pluie et de l'écoulement de surface dans les cours d'eau pendant la crue affecte certains groupes fonctionnels du sol en amont, comme les groupes liés au cycle de l'azote.

Pour soutenir et confirmer les résultats des investigations *in situ*, j'ai également mené une expérience en microcosme pour évaluer la réponse de la communauté bactérienne lors de l'ajout de l'écoulement de surface du sol sous différentes LU (2YF, 8YF), TW et TWO pendant 96h d'incubation. Les résultats de cette expérience ont confirmé que le changement de structure de la communauté bactérienne se produisait en fonction des différents traitements en ajoutant un écoulement provenant de différents sols. La richesse et l'équitabilité de la communauté bactérienne dans les microcosmes traités ont diminué après 96h d'incubation. La concentration en DOC était significativement liée à la structure de la communauté bactérienne dans différentes UT pendant les 96h d'incubation. Comme pour

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Les résultats de mes travaux montrent l'importance de prendre en compte à la fois l'UT passées et présentes, ainsi que les processus hydrologiques lors de l'évaluation de la diversité et des fonctions microbiennes des cours d'eau. Alors que les expériences en conditions contrôlées (micro et mésocosmes) ont permis de distinguer l'importance relative des écoulements terrestres directs et de la communauté du sol sur la structure bactérienne des cours d'eau, l'approche *in situ* a permis de souligner la nécessité d'utiliser des pratiques de gestion durables d'UT si nous souhaitons atténuer les impacts sur les systèmes aquatiques en aval.

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Abbreviations

AMOVA	Analysis of molecular variance
ANOSIM	Analysis of similarity
ANOVA	Analysis of variance
C1	Component 1 of PARAFAC
C2	Component 2 of PARAFAC
C3	Component 3 of PARAFAC
CCA	Canonical correlation analysis
CDOM	Colored dissolved organic matter
DNA	Deoxyribonucleic acid
DOC	Dissolved organic carbon
DOM	Dissolved organic matter
EEM	Excitation-Emission Matrices
Em	Emission
Ex	Excitation
FI	Fluorescence index
FL	Free-living
HIX	Humification index
HZ	Hyporheic zone
LU	Land use
NDMS	Non-metric multidimensional scaling
OTU	Operational taxonomic unit
PA	Particle attached
PLS	Partial least squares
POC	Particle organic carbon
S	Spectral slope
SOC	Soil organic carbon

SUVA ₂₅₄	Carbon-specific absorption at 254 nm, index of the aromaticity of DOC
TN	Total nitrogen
TOC	Total organic carbon
TP	Total phosphorous
TSS	Total suspended solids
TW	Teak plantation with understory
TWO	Teak plantation without understory
Sr	Slope ratio between UV wavelength region (275-295 nm) and UV wavelength region (350-400nm), index of molecular weight
UR	Upland rice
2YF	Two year fallow
8YF	Eight year fallow

INTRODUCTION

Land use (LU) management with long – term sustainable practices is becoming a global challenge. LU change is a global phenomenon and the negative consequences of non-sustainable LU change and agricultural practices are becoming increasingly, particularly in developing countries of the intertropical zone. Shifting cultivation with long periods of fallow used to be the most prominent farming system in Southeast Asia (Heinimann et al., 2017). Due to high population growth combined with poverty of local population, crop expansion and agricultural intensification occurred rapidly. Shifting cultivation is replaced by other forms of agricultural such as tree crops, permanent agriculture (rubber tree, oil palm, plantation timber, etc) or is maintained but with much shorter fallow durations (Schmidt-Vogt et al., 2009). However, over the last twenty years, often as a consequence of government policy and economic drivers, the transition from shifting cultivation to a more permanent agriculture has become more and more prevalent (van Vliet et al., 2012).

LU change and agricultural practices play an important role in global carbon and nutrient cycles. LU activities affect not only terrestrial processes but also aquatic systems in catchment. LU change can disrupt carbon and nitrogen cycles, alter carbon storage in soils (Houghton and Goodale, 2004) and induce shifts in microbial community composition and function (Ye et al., 2009; Francioli et al., 2014). The transition from shifting cultivation to other land use declines soil fertility and accelerates soil erosion (van Vliet et al., 2012). Agricultural management practices (e.g. tillage, organic amendment addition, chemical fertilization, overgrazing, and reduced crop rotation) can also impact soil organic carbon concentrations and the composition and activity of the soil microbial community (Muhammad et al., 2014; Wei Tian, 2015a; Tian et al., 2017). Moreover, LU changes alter hydrological processes increasing flood magnitude and frequency due to changes in the runoff – evaporation balance. This increases soil erosion and sediment export from the soil surface into adjacent waterways due to higher runoff (Allan et al., 1997; Allan, 2004; Wang et al., 2012).

LU change in catchments impacts the pattern of microbial diversity and the composition of downslope water systems through the export of the bacterial community and soil organic carbon from terrestrial soils to streams that changed the environmental conditions at the local scale (Belt et al., 2007; Crump et al., 2012; Fazi et al., 2013). Becker et al. (2017) found that a substantial portion of organic matter is derived from the surrounding terrestrial ecosystem and serves as source of energy for aquatic bacterial community in stream ecosystems. Changes in source of dissolved organic carbon (DOC) or the ratio of terrestrial (allochthonous) or aquatic derived (autochthonous) organic matter can impact aquatic bacterial community structure and function (Crump et al., 2003; Kritzberg et al., 2006b; Perez and Sommaruga, 2006). This is due in part to autochthonous DOC that is enriched in protein and labile polysaccharides, and is therefore more labile, whereas terrestrial DOC consisting of more humic substances is more recalcitrant to bacterial degradation (del Giorgio and Davis, 2003).

Moreover, changes of hydrological conditions such as rainfall, runoff and stream flow can affect the dispersal of the bacterioplankton community and the export of allochthonous and autochthonous organic matter (Crump and Hobbie, 2005; Chiaramonte et al., 2013; Fazi et al., 2013). For example, temporary stream fragmentation due to seasonal variations decreases microbial dispersion and changes the quantity and quality of the DOC that, in turn, influence bacterioplankton community composition (Fazi et al., 2013). Yet, the effects of seasonal variation on stream bacterial community and characteristic of DOC has been much less studied in tropical systems.

Some studies from temperate and Arctic mountain systems has demonstrated the importance of biogeochemical conditions of both soil organic matter and soil microbial communities in structuring stream microbial populations and highlighted the role of stochastic processes and disturbance events *e.g.* hydrological pulses (Judd et al., 2006; Portillo et al., 2012). Thus, research on the land – water transfer of microorganisms through the hydrological network is of importance for both land use and aquatic system management, especially in upland catchment systems. Despite this, few works has focused on the impacts of LU change and agricultural practices on soil erosion and eroded organic carbon in runoff and downstream biodiversity in tropical systems (Janeau et al., 2014; Trinh et al., 2016). Moreover, to my knowledge, none of this work has looked at the impact of LU and agricultural practices on the transfer of organic matter and microbial communities and their knock-on effects on in-stream microbial community structure. Nor have they attempted to identify potential indicators (*e.g.* DOM, microbial diversity patterns) of a disturbed landscape.

OBJECTIVE AND HYPOTHESIS

This thesis forms part of a larger research project (ANR Agrobiosphere Teciteasy) that aimed to clarify whether a typology of aquatic bacterial assemblages could serve as a proxy of the state of agro-ecosystems and their dynamics. In particular, my work aims to link how human-related environmental changes in terrestrial agro-ecosystems affect the metabolic capabilities and taxonomic diversity of aquatic bacteria, – *i.e.* free-living (FL) or particle attached (PA) cells, in subjacent freshwaters. To achieve this goal, aquatic microbial communities and physical-chemical parameters were measured i) in aquatic mesocosms impacted by runoff water from differently amended soil plots; ii) in aquatic microcosm experiment receiving overland flow from different land-use in Houay Pano, Laos; iii) in stream water during dry and wet seasons in the M-Tropics (formerly MSEC) observatory for environmental research located in the tropical watershed of Houay Pano, Laos; and iv) in runoff water from simulated rains on plots subject to a gradient of land-use in Houay Pano, Laos. High throughput sequencing of 16S rDNA genes provided detailed taxonomic profile of aquatic bacterial communities present in both water fractions (FL and PA), and for soil samples in the rain simulation experiment. Metabolic capacities were systematically assessed using Biolog[®] Ecoplates. These results were associated to chemical and optical measures of dissolved organic matter to disentangle the impacts of soil

outflows from various land use from natural seasonal variations in driving bacterial diversity, distribution and general functioning. Soil microbial diversity was also assessed in samples exposed to rain simulation to measure the relative leakage of specific taxonomic groups and associated potential functions from soil into runoff waters.

The main question of my thesis is **how LU change, its legacy and agricultural practices affect aquatic bacterial diversity and functioning in a tropical system.**

To answer this question, we tested the following three hypotheses:

- **Hypothesis 1:** Stream bacterial community is strongly driven by vicinal LU and its legacy as well as agricultural practices
- **Hypothesis 2:** Terrestrial runoff from different LU accelerates the transfer of the soil bacterial community into stream
- **Hypothesis 3:** DOC of terrestrial origin controls aquatic bacterial community diversity and structure, especially for the PA fraction as compared to the FL fraction

THESIS STRUCTURE

Figure 0 provides a general overview of the thesis structure.

Chapter 1 provides the general literature review of the thesis.

Chapter 2 gives a detailed description of the study areas and sampling protocol. This part also describes the general methodology of the five experiments. A controlled mesocosm experiment was conducted in 2012, in the Dong Cao catchment, Vietnam. *In situ* sampling, rain simulation and microcosm experiments were conducted in the Houay Pano catchment, Laos in 2014, 2015 and 2017 respectively. This thesis hence presents and discusses the results of different experiments at two different scales: mesocosm/microcosm experiments and *in situ* investigation. The mesocosm experiments are disclosed in **Chapter 3**. This chapter focuses on understanding the direct response of aquatic bacteria to terrestrial runoff from different amended soils. It gives a detailed view about changes of DOC and bacterial diversity and metabolic capacity to an addition of runoff from soils amended with different organic fertilizers to lake water in a mesocosm experiment.

To better understand the effects of terrestrial runoff from different land uses within a complex hydrological network in more realistic conditions, we conducted *in situ* investigations and soil and water sampling in a mixed land use tropical montane catchment in Houay Pano, Laos. **Chapter 4** examines the changes in DOC and bacterial communities transferred from soil under different land covers into runoff water with rain simulation experiment. Then in **Chapter 5**, I develop two sections to assess how terrestrial runoff influences downstream systems. First, we examined how LU change and its legacy impacts the concentration and characteristics of DOC and aquatic bacterial metabolic capacity and diversity along the stream at base flow during two, typical, tropical seasons. The second section presents

the results of the sampling of a flood event at a series of sites along the stream in order to determine how runoff from different LU affects DOC and bacterial export to the stream at high flow as compared to base flow. **Chapter 6** provides preliminary results from the microcosm experiment in regards to the results from the *in situ* investigations. This chapter assessed the response of stream bacterial community when directly exposed to overland flow from different LU into stream water. Lastly, **Chapter 7** summarizes the main results obtained throughout the thesis, discuss those in the context of the rapid, ongoing LU change in the humid tropics and highlights the different perspectives of this work.

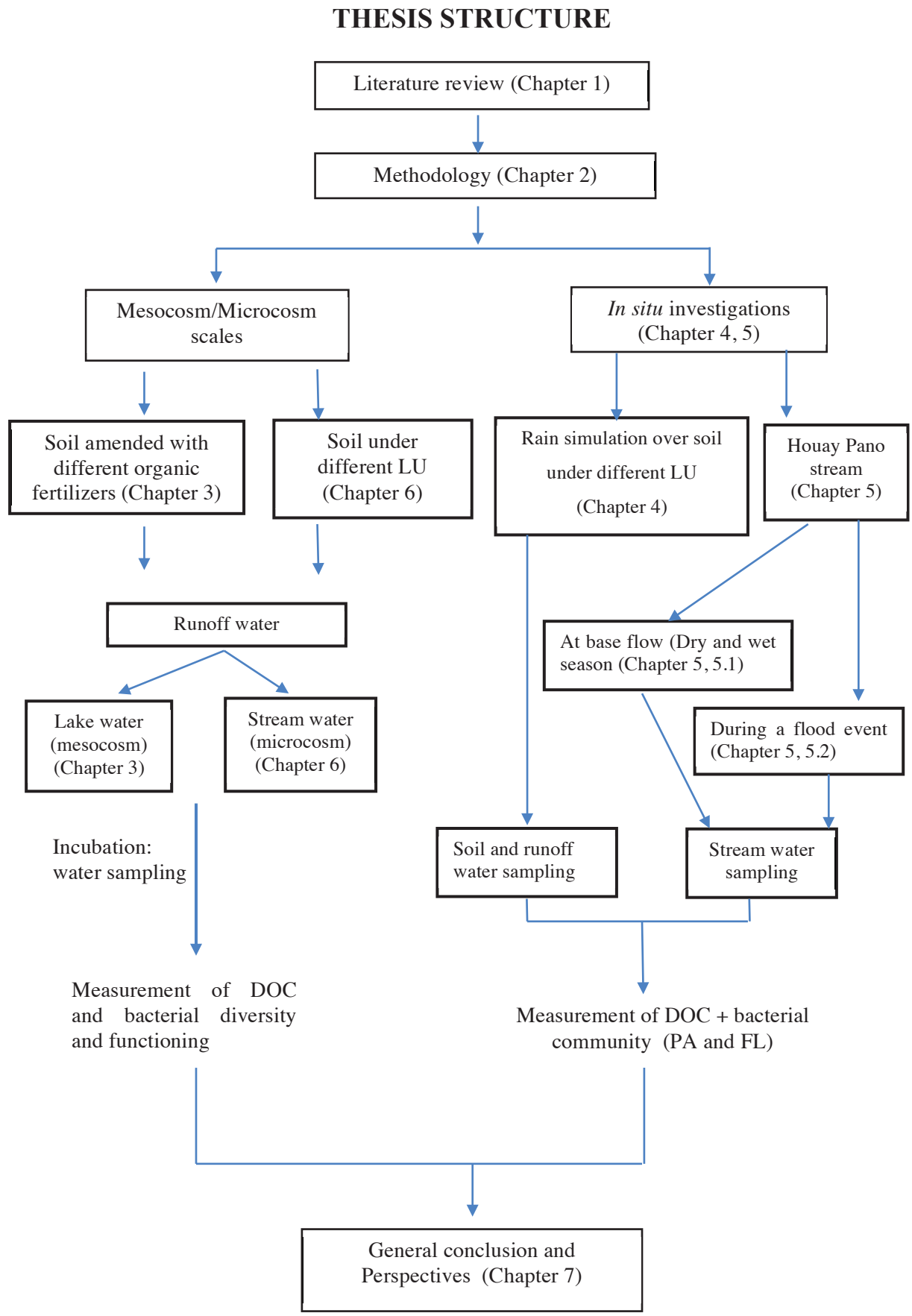


Fig.0: General overview of the thesis structure

CHAPTER 1

LITERATURE REVIEW

1.1. Land use and land cover

1.1.1. Definition and transition

Land use (LU) refers to human activities such as the conversion of natural landscapes for human consumption or land management practices. This includes clearing forests, practicing intensive agriculture, shifting cultivation and expanding urbanization (Foley et al., 2005). LU activities occur to satisfy human demands for food, fiber, timber, shelter and other ecosystem goods.

The transition stages of LU from natural ecosystems to frontier clearings, then to subsistence agriculture and small-scale farms and finally, intensive agriculture and urbanization vary widely across the globe, as a function of their history, social, and economic conditions, and ecological characteristics (DeFries et al., 2004; Foley et al., 2005) (Fig 1.1). In particular, LU transition may occur from a system dominated by annual crops for local consumption to a system with large tree plantations in response to market demand (Lambin and Meyfroidt, 2010).

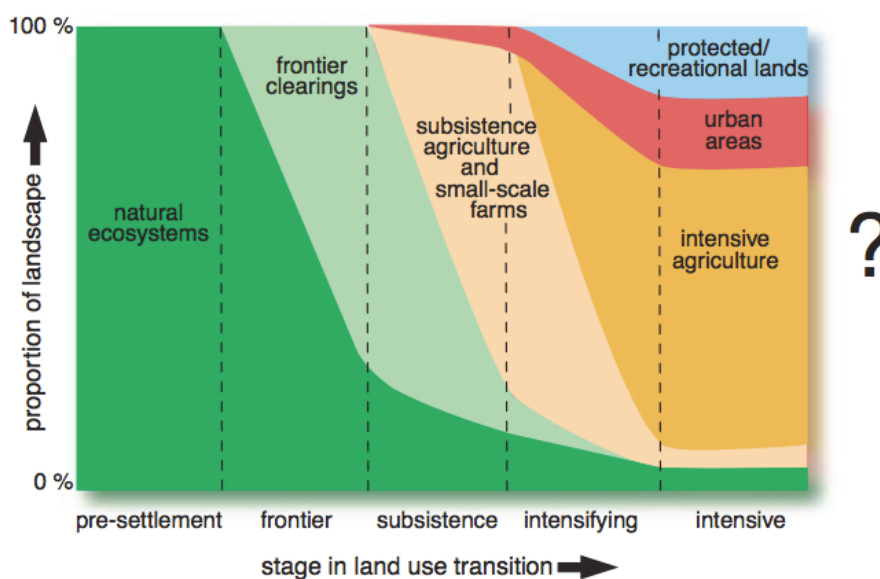


Fig 1.1: Land use transition. Source: Foley et al. (2005)

Forest transition

A forest transition is defined as a change in land cover trend, which is viewed as one of the important process in LU transition system (Lambin and Meyfroidt, 2010). Particularly, the concept of forest transition concerns the change of national forest areas from decreasing to expanding at a national or regional scale. The forest transition from deforestation to reforestation has varies in developed and developing countries (Lambin and Meyfroidt, 2010). According to the Global Forest Resources

Assessment 2000, the world's natural forest area decreased annually by 16.1 million hectares during the 1990s, which accounted for 4.2% of the total natural forest in 1990 (FAO, 2001). However, by the middle of the twentieth century, deforestation had essentially decreased in the world's temperate forest as a consequence of the post-industrial economy based on the service sectors (FAO, 2012). Due to economic expansion, the labor force is driven from agriculture to other economic or service sectors and from rural to urban areas. The forest transition from decreasing to expanding forest density occurred mostly in Europe and in North America (Lambin and Meyfroidt, 2010). Nevertheless, the net global decrease in forest area was estimated at 9.4 million hectares per year over the decade from 1990 to 2000 (FAO, 2001). The forest transition of tropical regions also decreased. The FAO, 2010 estimated that 15.2 million hectares on average of tropical forest were lost per year during the 1990s. The FAO, in 2010, reported that the largest area of forest that was cleared in the world during 1990 – 2010 occurred almost exclusively in tropical regions (FAO, 2010) (Table 1.1).

Table 1.1: Annual change in forest area by region and sub-region, 1990-2010. Source: FAO, 2010

Region/sub-region	1990-2000		2000-2010	
	1000 ha/year	%	1000 ha/year	%
Eastern and Southern Africa	-1841	-0.62	-1839	-0.66
Northern Africa	-590	-0.72	-41	-0.05
Western and Central Africa	-1637	-0.46	-1535	-0.46
Total Africa	-4067	-0.56	-3414	-0.49
East Asia	1762	0.81	2781	1.16
South and Southern Asia	-2428	-0.77	-677	-0.23
Western and Central Asia	72	0.17	131	0.31
Total Asia	-595	-0.10	2235	0.39
Russian Federation (RF)	32	n.s.	-18	n.s.
Europe excluding RF	845	0.46	694	0.36
Total Europe	877	0.09	676	0.07
Caribbean	53	0.87	50	0.75
Central America	-374	-1.56	-248	-1.19
North America	32	n.s.	188	0.03
Total North and Central America	-289	-0.04	-10	0.00
Total Oceania	-41	-0.02	-700	-0.36
Total South America	-4213	-0.45	-3997	-0.45
World	-8327	-0.2	-5211	-0.13

n.s. = insufficient data

Cropland expansion and agricultural intensification

Cropland expansion, the conversion from forest to cropland, is one of the most globally significant LU change (Foley et al., 2005). This is due to a combination of increasing food demand and market forces linked to global population growth. This increased production was made possible, in part, by the large scale clearance of major areas of forest: about 29% of forest and woodland was converted to agricultural uses from 1700 to 1992.

The rate of cropland expansion has slowed in the last three decades thank to agricultural intensification. Scientific and technological achievements have increased agricultural outputs through the use of high-yield crop varieties, chemical fertilizers, pesticides and herbicides and, irrigation systems (Matson et al., 1997). For example, tropical Asia increased its food production mainly by increasing fertilizer uses and irrigation. Agricultural intensification is expected to decrease the demand for cropland, thereby reducing the rate of conversion of natural ecosystems. This could lead to a stabilization of this LU transition or even an increase in tree cover.

In developed economies, a recent trend towards the conversion of agricultural land into non-agricultural uses has emerged. This transition towards urbanization, the abandonment of agricultural activity and the conversion of arable land or of permanent crops to other uses (pasture, woodland) is particularly evident in Western Europe or in the Northeastern United States where decreased in cropland have been observed (Lambin et al., 2003).

Urbanization

Urbanization also affects LU change through the transformation of the urban-rural linkage. The phenomenon of rural migration towards urban centers is driven by the perceived economic opportunities and by market forces (Satterthwaite et al., 2010). The rapid transition from rural to urban populations demands more resources and living spaces. Expanding cities are responsible for LU transitions such as forest clearing and the reduction of agricultural land surface to provide the surface needed for the establishment of the infrastructure required to support a growing population. Urbanization was in the past a prevalent phenomenon in developed countries (Glaeser, 2003). It is now almost complete; i.e. almost 80% of Europeans already live in urban areas (Haase et al., 2014). However, in developing economies urbanization continues to occur rapidly. Indeed, urban areas in developing economies are projected to increase from 300,000 km² in 2000 to 770,000 km² in 2030 and 1,200,000 km² in 2050 (Angel et al., 2011).

1.1.2. Specifics of tropics and rural areas in developing countries

LU in rural tropical areas is rapidly changing from natural forest to perennial or annual crops (Ribolzi et al., 2016). High rates of tropical deforestation in developing economies are strongly linked to population growth and poverty. Growing population means increased demand on food productivity and increased demand for land for annual or permanent crops. Poor agricultural intensification and development increase pressure to convert forests and other marginal lands to crop production (Barbier, 2004). As a consequence, land expansions are expected to be higher in the agricultural sectors of developing economies in tropical regions.

In Asia, 65 % of forest loss was a result of LU change to agriculture (FAO, 2009) with 23% of this loss resulting directly from intensification of slash and burn agriculture, and 13% from direct LU conversions to small-size farms (FAO, 2009). In the early 20th century, about 90% of Southeast Asia was

covered by forest and approximately 5% of the total land area was covered by cropland (Tao et al., 2013). From 1901 to 2000, the cropland area increased rapidly while forest area decreased. By 2005, forest area had decreased by 15.8% and cropland increased nearly 3 times when compared with the rate of increase in the early 20th century in the Southeast Asia. Most of these changes in LU occurred in Thailand, Indonesia, and the Philippines (Fig 1.2) (Tao et al., 2013).

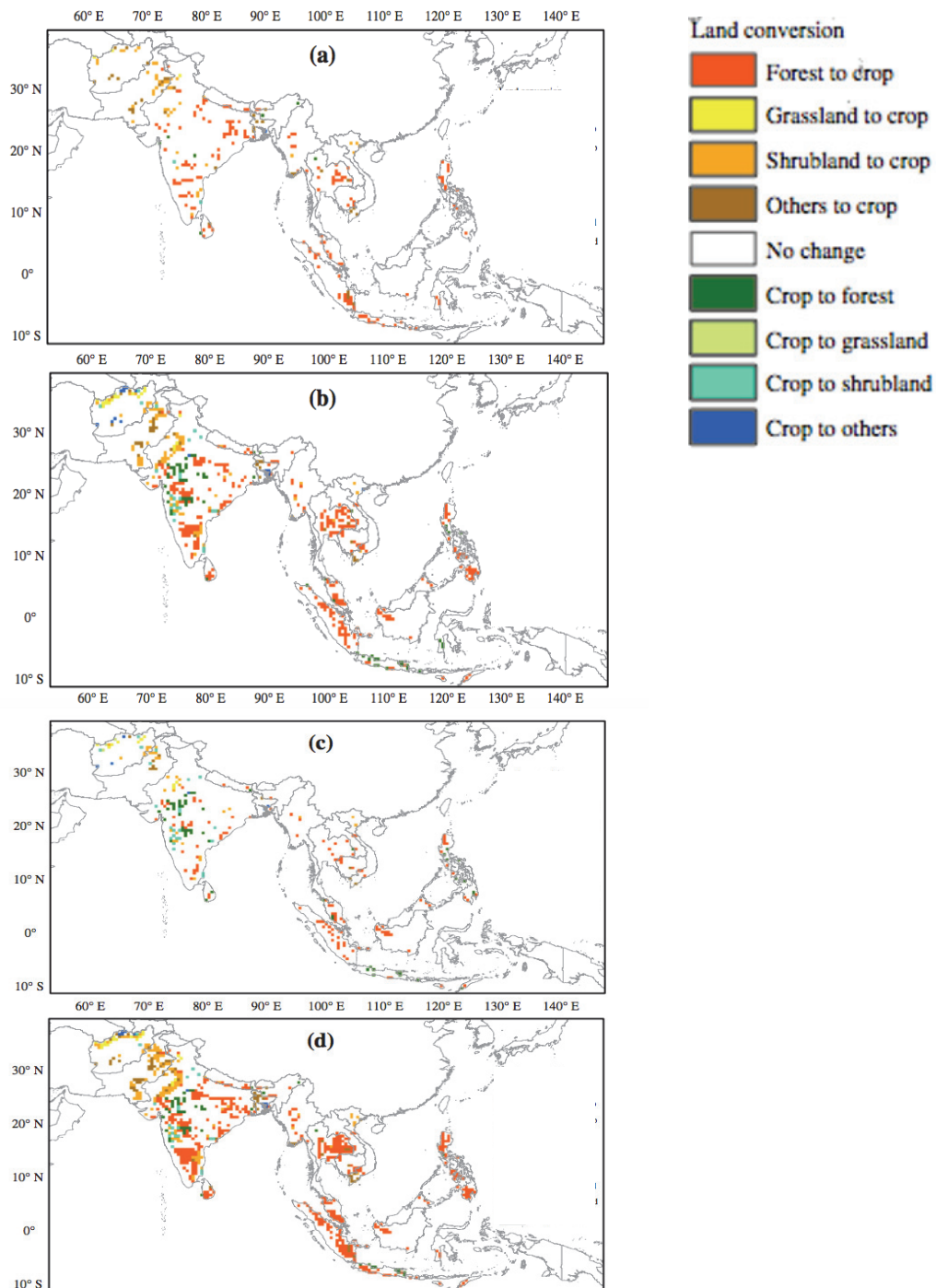


Fig 1.2: Historical land conversions in Tropical Asia for (a): 1901–1950, (b): 1951–2000, (c): 1981–2000, (d) 1901–2000. Source: Tao et al. (2013)

Fertilizer application is one of the most popular agricultural practices in rural tropical countries to improve soil quality and productivity. The average rate of nitrogen fertilizer use in cropland increased from nearly 0 in the early 20th century to 2.8 g N m² yr⁻¹ in 2005 in tropical Asia (Tao et al., 2013). The use of chemical and organic fertilizers influences not only the soil and plant growth but also impacts the surrounding environment. Chemical fertilizers are cheaper, have higher nutrient contents and are rapidly taken up by plants (Robertson and Vitousek, 2009). However, many studies have indicated that the excessive use of chemical fertilizers has led to a range of environmental problems, particularly nutrient loss, soil acidification or basification, loss of useful soil microbial communities (Pernes-Debuyser and Tessier, 2004; Geisseler and Scow, 2014; Wang et al., 2017b) and surface water and groundwater contamination (Hallberg George, 1987; Easton and Petrovic, 2004).

The need for sustainability in agricultural practices has meant there is a movement toward the use of organic fertilizers as an alternative to mineral fertilizers. Organic amendments, including animal manure, solid wastes and various types of compost, biochar, are considered to improve soil quality and productivity (Glaser et al., 2014; Greenberg et al., 2017). Compared to chemical fertilizers, organic manure has lower nutrient contents and is often not optimized in terms of NPK concentrations and ratios (Gupta and Hussain, 2014). However organic fertilizers bring other benefits to soils due to maintenance of soil organic carbon, increasing soil microbial activity, improving soil structure and root development, and increasing soil water availability (Pernes-Debuyser and Tessier, 2004).

1.2. Consequences of land use change

LU activities impact biogeochemical cycles, cause biodiversity loss, modify and fragment habitats, degrade soil and water quality, and over-exploit native species. In streams, LU change causes more erratic hydrology and increased contaminant concentrations and affects ecological interactions (Lear et al., 2009).

1.2.1. Physical/ Hydrological perspective

One of the prominent consequences of shifting LU in tropical catchment systems is to increase soil erosion and overland flow that affects water quality in streams and rivers (Valentin et al., 2008).

Rainfall – runoff is an important component of hydrological cycles. Overland flow (surface runoff) occurs when soils become saturated, which is common in low lying areas, or if precipitation exceeds the infiltration capacity of the soil (Kuchment, 2004). Raindrop impacts cause compaction of bare soil and increase soil particle detachment (Battany and Grismer, 2000; Mohamadi and Kavian, 2015). Surface roughness is one of the important factors affecting soil surface storage and infiltration, consequently controlling runoff and soil erosion processes during the rainfall (Ding and Huang, 2017). Differences in surface roughness can be due to soil texture and agricultural practices, as well as the deposition of residues or plant debris. Surface cover such as living vegetation or debris can reduce the

impact energy of the raindrop, preventing the formation of surface crusts and enhancing infiltration capacity and hence reducing soil erosion (Battany and Grismer, 2000; Nunes et al., 2011).

Overland flow along with deep drainage (leaching) and subsurface drainage are the main pathways for the export of contaminants from land to water (Fig 1.3). The delivery of pollution to stream from hillslopes depends on the spatial and temporal movement of water via flow pathways and their connectivity (McKergow et al., 2007).

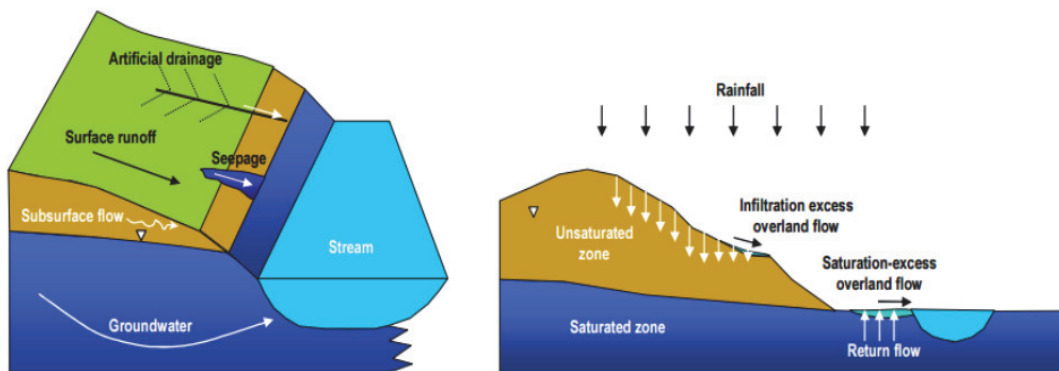


Fig 1.3: Water pathways from pasture to stream (left) and surface runoff generated by infiltration-excess and saturation-excess overland flow (right). Source: McKergow et al. (2007)

Soil erosion is a natural process involving the detachment and transport of soil particles by agents such as rainfall, runoff, wind, etc., (Lal, 2003). The soil erosion process is comprised of four stages: i) detachment of particles, ii) breakdown of aggregates, iii) transport and redistribution of sediments over the landscape and iv) deposition in aquatic ecosystems (Lal, 2003). The transfer of solute from the soil surface to overland flow includes the transfer of solutes from the soil surface by diffusion induced by the concentration gradient, ejection of solution from the soil surface by raindrops, erosion by raindrops and surface flow of sediment with adsorbed chemicals, and adsorption-desorption of the adsorbing chemicals (Fig 1.4) (Shi et al., 2011).

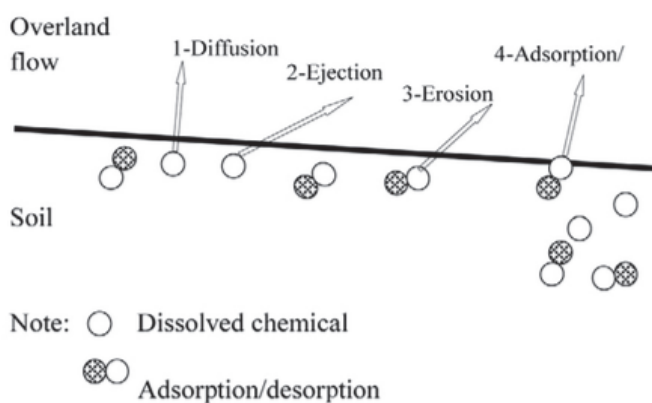


Fig 1.4: Process of solute transfer from soil surface to overland flow. Source: Shi et al. (2011)

Overland flow, and hence the rate of soil erosion as well as solute transfer processes from soil surface to overland flow are controlled by local site characteristics, particularly soil texture, structure and properties, vegetation type and degree of cover, topography and rainfall (Kuchment, 2004; Montgomery, 2007; Shi et al., 2011). Infiltration rate is closely related to soil texture. For example, coarse textured soils have higher infiltration rates than fine texture soils due to large and well-connected pore spaces. Topographical factors, including slope gradient and slope length, directly affect the potential energy and the nature of overland flow. Runoff volume increases with the increase of slope gradient and length (Shi et al., 2011). Vegetation and the degree of surface crusting also contribute to determine overland flow rates with high vegetation coverage and low crusting leading to higher infiltration as compared to bare soils with high crusting rates (Kuchment, 2004). The links between LU change and agricultural practices, soil erosion and overland flow have been found in many studies in both temperate and tropical regions (Montgomery, 2007; Valentin et al., 2008; Nunes et al., 2011; Lacombe et al., 2017). For example, Nunes et al. (2011) found that the soil loss by erosion for the cereal crop was more than 20 times higher than that of fallow land or short-term abandoned land. The formation of shrub cover and recovering oak forests resulting from land abandonment showed low rates of runoff (1.05 mm) and soil erosion (0.62 g m^{-2}) due to the high infiltration capacity compared to cereal crop with 77.7 mm of runoff and 627.1 g m^{-2} of soil erosion (Nunes et al., 2011).

Shifts towards different LU can cause changes in soil erosion, as well as loss of soil organic matter and nutrient concentrations (Navas et al., 2012). For example, the change of forest to maize crops caused the loss of soil organic carbon (SOC) by 20%, 36%, and 47% after three, seven, and ten years in eastern Thailand (Jaiarree et al., 2011). Niu et al. (2015) when evaluating soil erosion and variation in SOC, total nitrogen (TN) and total phosphorous (TP) contents under different LU types in the catchment of the Dianchi Lake of China found that grasslands and forestland experienced a lower degree of soil erosion and higher SOC, TN and TP compared to tillage or abandoned agricultural lands. Similarly, the conversion from native tropical forest (Kenya) and subtropical grassland (South Africa) ecosystems to agriculture led to the depletion of the total SOC (46-73%) and N concentration (37-73%) during the first 4 years of conversion (Solomon et al., 2007). Tiessen H (1992) reported that 30% of carbon, nitrogen, and phosphorous of soil were lost when applying slash and burn agriculture during 6 years in tropical countries. Other authors found that 8–10 years of fallow were needed to restore fertility levels to those similar to the original site conditions prior to cultivation (Matson et al., 1997). Similarly, the conversion from cultivated land to urban areas decreased organic production and nutrient cycling such as phosphorus and potassium due to the loss of vegetation (Song and Deng, 2015). Wang et al. (2017a) also indicated that LU change driven by urbanization influences the microbial processes involved in nitrogen cycling.

Soil erosion and overland flow processes that are accelerated by LU and agricultural practices are responsible for transporting sediments, carbon, nutrients and other chemical fluxes from soils to

surface waters (Kuchment, 2004). Hughes et al. (2012) found that mixed LU catchments export up to three times more sediment than the native forest catchments. Similarly, Huang and Lo (2015) indicated that the 6.9% decrease of forest land and 9.5% increase of agricultural land caused an increase in sediment yield of 0.25 t ha^{-1} over the whole river basin.

SOC is easily transported by runoff water due to its relative low density ($<1.8 \text{ Mg/m}^3$) and because it is concentrated in the vicinity of the soil surface (Lal, 2003). Leaching losses of dissolved organic carbon (DOC) may also be an important pathway of carbon loss from agricultural systems (Kindler et al., 2011). The overall mean DOC exported from the pasture and mixed (pasture, pine, and native) catchment were 50% higher than the native forest catchment (Quinn and Stroud, 2002). This loss of organic carbon from soils through erosion and runoff can have serious consequences for agricultural lands, as well as on the downstream water bodies to which it is imported (Cole et al., 2006). For example, Walmsley et al. (2011) found that carbon leaching from soils reduced potential soil carbon gains by $20 \text{ g m}^{-2} \text{ y}^{-1}$. Additionally, higher DOC concentration impacted the transport of nutrients and pollutants such as enhancing the sorption and mobility of pesticides and heavy metals (Veum et al., 2009; Pagano et al., 2014). This leads to degrade the drinking water quality. Moreover, intensive agriculture such as the excessive application of fertilizers has also become the largest source of excess of nitrogen and phosphorous to aquatic ecosystem (Matson et al., 1997; Bennett et al., 2001; Foley et al., 2005).

1.2.2. Sources, forms and characteristics of organic carbon

Sources and forms

Organic carbon in water can be classified into two categories: particulate ($>0.7 \mu\text{m}$) and dissolved ($<0.7 \mu\text{m}$) (Nachimuthu and Hulugalle, 2016). DOC is a general description of the organic carbon dissolved in water. Dissolved organic matter (DOM) is a term used generally for dissolved organic substances, but the term is often used interchangeably with DOC in the literature, despite the fact that DOC makes up a fraction of the DOM profile (Pagano et al., 2014). There are two sources of DOC in aquatic systems: allochthonous (external to the system) and autochthonous (internal to the system). Autochthonous DOC is released from primary production from macrophytes and benthic and phytoplankton (Thomas et al., 2009; Bauer et al., 2011) through different mechanisms including predator grazing, cell death, viral lysis and extracellular release (Azam et al., 1983). Meanwhile, allochthonous DOC is derived from terrestrial vegetation and soil. It is estimated that about 2.9 Pt C yr^{-1} of the terrestrial organic C enters freshwater systems worldwide (Tranvik et al., 2009). Terrestrial plants and soil organic matter are the major sources of carbon in temperate headwater streams (Vannote et al., 1980). The quantity and quality of allochthonous DOC that enters aquatic ecosystems is controlled by many factors such as the hydrologic conditions of the stream, LU management and agricultural practices, precipitation, soil type, vegetation cover, etc. (Willett et al., 2004). For example in New Mexico, USA, forest soils tend to lose more organic carbon than grassland soil, with on average 39 g and 5 g lost

respectively (Puttock et al., 2013). Williams et al. (2010) in the survey of 43 streams distributed throughout catchments of mixed LU in Southern Ontario, Canada also indicated that DOM in agriculturally affected streams was likely more labile and accessible to the microbial community than DOM in the wetland and forest affected streams.

Characteristics

DOC pool in a stream is a mixture of recalcitrant substances, particularly as humic and fulvic acids (Wallage, 2006) with labile compounds including carbohydrates, peptides, amino acids, small carboxylic acids and alcohols (Klavins, 1997). Humic substances are high molecular weight compounds, and make up to 50% of DOC. Humic substances contain a large proportion of fulvic acids and some humic acids. Fulvic acids are formed from the microbial degradation of plant and animals, are a mixture of weak aliphatic and aromatic organic acids, whereas humic acids are comprised of aromatic groups such as methoxyls and phenolics. The remaining of DOC includes 30% of macromolecular hydrophilic acids and about 20% of low molecular weight organic molecules such as carbohydrates, carboxylic acids and amino acids.

Stream DOC originates from different sources with unique chemical and spectroscopic properties (Kalbitz et al., 2003). Spectroscopic analyses including UV absorbance and fluorescence are used to identify the chemical characteristics of aquatic DOC. For example, the specific UV light absorption at 254 nm ($SUVA_{254}$) indicates the aromaticity of DOC (Weishaar et al., 2003) whereas the absorption at 280 nm shows the contribution of complex compounds to DOC (Kalbitz et al., 2003). The spectral slopes (S), determined over selected wavelength ranges such as between 275-295 nm or between 355-400 nm are surrogates for average molecular size and bioavailability and hence can provide information on the sources and diagenetic state of the DOM (Helms et al., 2008). The spectral slope ratio, S_r , determined as $S_{275-295} / S_{355-400}$, provides information on the apparent molecular weight of the DOM (Helms et al., 2008; Fasching et al., 2016).

1.3. Aquatic microbial community

Microorganisms play a key role in ecosystem functioning through their main contribution to biochemical degradation of organic matter in nutrient cycle. About 80% - 90% of the processes occurring in soils are mediated by bacteria (Nannipieri et al., 2003). Soil microorganisms and related nutrient cycling processes are important for soil formation and productivity (Geisseler and Scow, 2014; Li et al., 2015). In aquatic systems, microorganisms play an essential function in the transfer of energy and material. Microorganisms are also the major biological component involved in the decomposition and mineralization of DOM and significantly affect the fate of DOC (Chróst, 1990; Fenchel, 2008).

1.3.1. Free living (FL) and Particles Attached (PA) bacteria

In aquatic environments, bacteria can be separated in two groups: particle-attached (PA) and free

living (FL) fractions. The PA fraction is collected on a 3 - 20 μm filter, whereas the FL fraction is collected on 0.2 - 3 μm filter (Crespo et al., 2013). The PA bacteria are often larger, more abundant and have higher metabolic activity than the FL bacteria in the same environmental conditions (Acinas et al., 1999; Grossart et al., 2007). Some studies indicated that FL bacteria are more diverse than the PA fraction in oligotrophic marine systems (Acinas et al., 1999; Ghiglione, 2007; Li et al., 2015a), whereas the opposite pattern was found in some mesotrophic and eutrophic lakes (Parveen, 2011; Tang et al., 2015). However, other studies using pyrosequencing technology found higher diversity of PA bacteria compared to FL bacteria in lakes, rivers (Kellogg, 1993) and even in the sea (Crespo et al., 2013; Ortega-Retuerta et al., 2013).

The majority of studies have shown that the community structure of PA and FL differed in marine systems or in fresh water habitats (Acinas et al., 1999; Crump et al., 1999; Simon et al., 2002), but only a few studies indicated the similarity between two fractions such as in freshwater mesocosms (Riemann and Winding, 2001). The similarity and difference between PA and FL community structure probably depends on the origin and composition of the particles to which the bacteria are attached (Hollibaugh et al., 2000). Moreover, metabolic activities between PA and FL fraction was found different in some studies (Becquevort et al., 1998; Ganesh et al., 2015). For example, Ganesh et al. (2015) studying on the dissimilatory processes of the nitrogen cycle in the oxygen minimum zone of the Eastern Tropical North Pacific found bacterial gene counts mainly related to anammox-associated transcripts were 8 to 15 fold higher in the FL fraction than the PA fraction. The presence of particles was found to facilitate anammox and nitrification rates by providing ammonium remineralized by heterotrophic PA bacteria consuming particulate organic matter (Ganesh et al., 2015; Happel et al., 2018).

The studies on the PA and FL fraction bacterial communities have mainly focused on marine and estuarine systems or on oligotrophic and mesotrophic deep lakes in temperate regions (Zhang et al., 2007), but much less is known about the PA and FL fraction community structure in tropical freshwater systems.

1.3.2. Factors controlling microbial community

Microbial communities are driven by many factors and processes in the ecosystem. Abiotic or physical forces that form habitat conditions including disturbance, salinity, pH, oxygen concentrations, nutrient, etc. and biotic forces such as competition, facilitation, predation, commensalism and parasitism also regulate community composition (Findlay and Sinsabaugh, 2002; Fierer and Jackson, 2006; Zornoza et al., 2015). A key environmental factor, DOC, has a determining role in controlling aquatic bacteria community structure and functioning (Kritzberg et al., 2006b). In stream and river ecosystems, microbial communities vary temporally and spatially as a function of nutrient availability, temperature, organic matter quality and quantity, hydrological factors or LU (Zeglin, 2015).

The main factors and processes that affect bacterial diversity and biogeography in freshwaters includes local habitat conditions (species sorting) and regional factors such as dispersal and neutral assembly (Brendan Logue and Lindström, 2008; Lindström and Langenheder, 2012). But the latter factors are less frequently studied in contrast to the first (Fig 1.5). In aquatic systems, bacteria communities can disperse via water flow from terrestrial soil into streams such as overland flow. At high flow, especially during the storm events, dispersal becomes an important mechanism for bacterial community structure. However, species sorting is predominant mechanism if the dispersal is low (Adams et al., 2014). Lindström and Östman (2011) also found that high dispersal rate can affect bacterial community composition and functioning, but this effect also depends on the characteristic of the habitats into which bacteria disperse. Soil bacterial communities can immigrate and persist in aquatic systems such as streams if a large number of cells disperse to that habitat and are able to outcompete native communities.

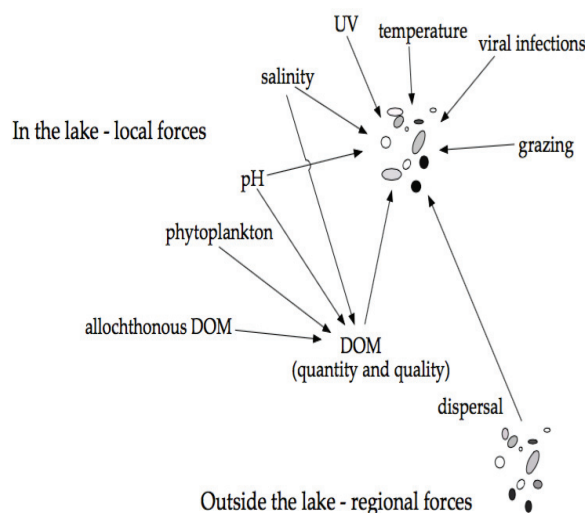


Fig 1.5: Different factors affecting bacterial communities in inland water. Source: Brendan Logue and Lindström (2008)

1.3.3. Microbial degradation of DOC

DOC plays an important role as substrate and energy sources for heterotrophic microorganisms. The degradation and mineralization of DOC through bacterial activities may significantly affect the fate of DOC in aquatic systems. There are two main metabolic pathways in bacterial cell: bacterial respiration (catabolism) and bacterial production (anabolism). Bacterial production is the process of consuming energy and carbon substrates to produce biomass. Respiratory activities of heterotrophic bacteria may turn organic C into inorganic C, completing the C cycle. The final turn of this cycle is termed mineralization.

The composition of DOC is divided in two types, based on bacterial reactivity as followed: the

refractory (R-DOC) and labile DOC (L-DOC). R-DOC is subdivided into fractions based on their lifetime: semi-labile (~ 1.5 years), semi-refractory (~20 years), refractory (~16 000 years) and ultra-refractory (~40 000 years) (Hansell et al., 2012). L-DOC is efficiently taken up by bacteria, therefore the turnover time is very short (from hours to days). Molecular weight or size is also an important factor affecting the microbial utilization of DOM. For instance, high molecular weight DOM is considered more available for bacterial uptake than low molecular weight DOM (Amon and Benner, 1994). In the study of biological lability of stream water fluorescent dissolved organic matter (FDOM) from a temperate stream, Cory (2012) indicated that humic-like FDOM was more recalcitrant and more hydrophobic while Tyrosine-like and Tryptophan-like FDOM components contained both labile DOM, along with semi-labile and more recalcitrant moieties.

Microbial metabolism of DOC is controlled by environmental conditions such as temperature, availability of oxygen and inorganic nutrient and source and chemical structure of DOC (Scofield et al., 2015). Some studies found that labile DOM sources, such as organic fertilizer runoff, and autochthonous DOC are readily decomposed by microbes (Cole et al., 1988). In contrast, allochthonous DOC including larger aromatic macromolecules such as humic and fulvic acids derived from lignified plant material are considered to be more recalcitrant (Farjalla et al., 2006). However, Fonte et al. (2013) investigated the effects of a mixture of DOC sources on bacterial growth efficiency and concluded that the combination of DOC sources results in a more efficient biological use of carbon.

1.3.4. Links between bacterial diversity and functioning

The relationship between bacterial diversity and ecosystem functioning has mainly been studied as regards to specific nutrient cycles or processes such as nitrogen cycling processes (Bernhard and Kelly, 2016) or the type of organic matter utilized (Findlay and Sinsabaugh, 2002). The diversity of functional genes related to specific environmental conditions (Bernhard and Kelly, 2016) and microbial composition may reflect the metabolic potential of the community (Li et al., 2012). In general, the link between bacterial diversity and ecosystem functioning was found positively, negatively or neutral (Bond and Chase, 2002; Roger et al., 2016). Moreover, some studies have shown that broad functions of microbial ecosystems such as respiration in soil, nutrient uptake or biomass synthesis remained unchanged and independent of diversity due to high redundancy on these functions (Griffithsa et al., 2001; Langenheder et al., 2005; Peter et al., 2011). In contrast, specific functions such as carbon mineralization, DOC degradation and enzyme activities are more sensitive to a reduction of diversity and changes in bacterial structure (Griffiths et al., 2000; Langenheder et al., 2006).

Regarding organic carbon degradation by microbes, this function specific to bacteria is susceptible to change with microbial diversity. Hernandez-Raquet et al. (2013) demonstrated that the high bacterial diversity of activated-sludge community increased the communities' ability to mineralize phenanthrene, a polycyclic aromatic hydrocarbon. Similarly, Peter et al. (2011) indicated that the rate of chitin and cellulose degradation depended on the presence of a single phylotype. However, the change of

microbial diversity also affects redundant functions that are carried out by more species. For example, Baumann et al. (2012) found that microbial diversity also affected redundant functions of soil organic carbon decomposition including some specific to wheat sugar decomposition (arabinose, xylose) or catabolism of the total sugar-C pool. In freshwater ecosystem, Ylla et al. (2013) found the reduction in stream and lake bacterial diversity affected the activities of extracellular enzymes involved in DOC degradation. The degradation of complex pool of organic matter required the interplay of diverse extracellular enzymes, showing the importance of bacterial diversity on multifunctionality.

In the literature, there is a lack of knowledge about the transfer of bacterial communities and organic carbon from soil into aquatic systems as a consequence of LU change and agricultural practices in a tropical catchments, and whether stream bacterial diversity and organic carbon are governed by terrestrial runoff from different LU, agricultural practices or even the legacy of LU change in upland soils. Which LU causes more soil degradation and loss of organic carbon and soil bacteria into the stream through overland flow? Moreover, the level of effect of LU change on stream bacterial community may differ as a function of the seasonal changes in hydrological flow (stream flow and overland flow). Therefore, my thesis aims to assess the effects of LU change and agricultural practices on bacterial diversity and metabolic capacity and organic carbon across terrestrial runoff and stream water. The thesis is expected to fill the gap of knowledge about the link between terrestrial and aquatic systems concerning the bacterial community of a tropical catchment that is subject to rapid LU change.

CHAPTER 2

METHODOLOGY

2.1. Study sites

2.1.1. Dong Cao catchment

Dong Cao catchment is located on the sloping area of Tien Xuan commune, Thach That district of Ha Noi, Vietnam ($20^{\circ} 57' 30''$ N, $105^{\circ} 29' 15''$ E; elevation 300 m a.s.l) (Fig 2.1). This catchment has an area of 50 ha and is bisected by a small stream that is connected to a downstream reservoir (volume of $700\,000\text{ m}^3$). The climate in the area is a monsoon climate with 80% of the rainfall occurring in the rainy season from April to October. The twelve year (1999 - 2011) mean annual precipitation measured in this sub-catchment was 1502 mm y^{-1} with a minimum of 1262 mm y^{-1} in 2010 and a maximum of 2506 mm y^{-1} in 2001 (Podwojewski et al., 2008; Tran et al., 2011). The mean slope of the area is 45% and the steepest slopes of around 120% are situated in the middle part of the basin which is the main cultivation area.

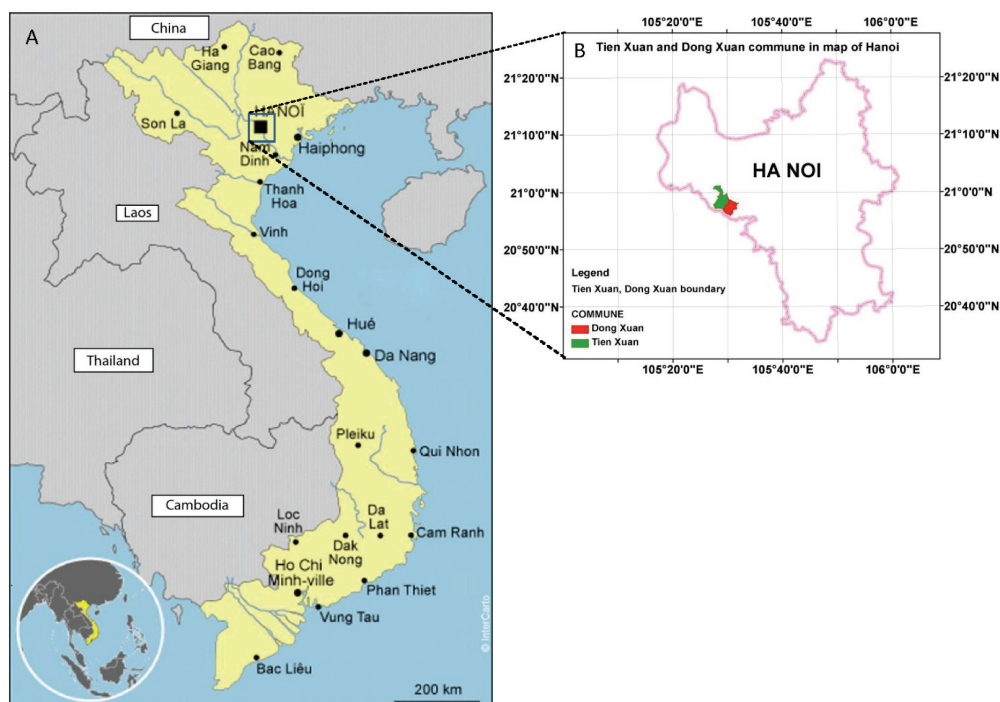


Fig 2.1: A) Map of Vietnam, B) Location of the Dong Cao catchment. Source: Janeau et al. (2014)

History of LU

Before 1970, the watershed was covered by primary forest. In the early 1980s, almost all the forest area disappeared due to deforestation. Since 1986, annual crops such as maize were cultivated in

this area, followed by cassava as the most important crop. From 1998, farmers were encouraged to plant trees such as *Eucalyptus* s.p, *Acacia mangium*, and *Vernicia montana* on steep slopes in accordance with government policy (Podwojewski et al., 2008). In 2001, cassava (*Manihot esculenta*) plantation was a main crop accounting for more than 50% of the total watershed area, along with old fallow and secondary forest. As a consequence of decreasing yields, cassava cultivation had almost disappeared by 2006. This decrease was accompanied by an increase in tree plantations, planted fodder and fallow lands. From 2009 to 2013, cassava and maize production covered annually 1.8 ha, corresponding to 3.5% of the total surface, planted forest (e.g. *Acacia mangium*) covered 11.9 ha (23.7%) and buildings and other permanent construction covered 1.0 ha (2%). The remaining 35.3 ha (70.7%) was covered by fallow (Janeau et al., 2014).

2.1.2. The Houay Pano catchment

The Houay Pano catchment is located in the uplands of the lower Mekong in Luang Prabang province in Northern Laos (Fig 2.2). The catchment covers 60 ha and is a sub-basin of the Houay Xon system, itself a tributary of the Mekong River. The regional climate is classified as tropical monsoon climate with heavy rainfall during the summer monsoon from mid-May to mid-October. The average annual precipitation in this area is 1300 mm year⁻¹. The geological substrate consists of perlites, sandstones and greywackes. The soils developed on these bedrocks are mainly Entisol, Ultisol and Alfisol (Soil Survey Staff, 1999; Evrard et al., 2016). The terrain is steep with a mean slope gradient of 54%, ranging from 0% to more than 171%.

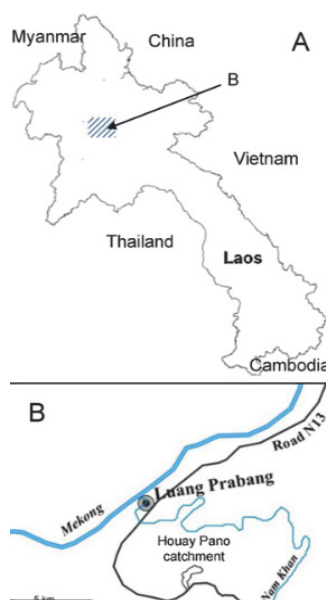


Fig 2.2: A) Location of Laos in South East Asia, B) Location of the Houay Pano catchment and the Nam Khan river. Source: Huon et al. (2013)

History of LU

The Houay Pano catchment encompasses several sub-basins that are subjected to various LU. LU in the catchment is representative of the rapid changes occurring in this region with a move from shifting cultivation towards teak tree plantations.

Prior to 1967, the catchment was covered by high forest with patches of disturbed forest. In 1967, approximately 24% of the catchment area was deforested for rice cropping with further 20% cropped by 1972. By 1982, 49% of the area including high forest and secondary forest was cleared. Until the end of the 1990s, short rotations of shifting cultivation prevailed, comprising 1-year cropping period followed by 8 years of fallow (Huon et al., 2013). The land area available for cultivation decreased due to rapid population growth, which led to an alteration in the traditional shifting cultivation systems with a doubling of cropping (two consecutive years) and a shortening of fallow to 1 to 4 years. In 1998, upland rice was the dominant crop in this catchment. Since then, farmers have diversified their production with cash crops such as Job's tears and maize. The rotation of shifting cultivation has now changed to a cycle of a 2-year cultivation period followed by a 2-year fallow period.

The first teak plantation was introduced in 1986 (Huon et al., 2013). Up to 2007, the percentage of catchment area covered with teak plantations remained low at only 4%. From 2008, it continuously increased to reach a maximum of 36% of the catchment area in 2014. An important change in LU from shifting cultivation towards teak plantations has been observed since 2011. Accordingly, in the work presented in this thesis, LU has been divided into two sub-periods. The first from 2002 to 2007 is characterized by the alternation between upland rice, Job's tear, annual crops and bush fallow, in addition to sparse teak plantations. The second sub-period from 2008 to 2015 is characterized by the synchronous expansion of teak plantations and reduction of annual crops (Fig 2.3). The last five years of this second sub-period are characterized by teak plantations of more than 3 years old with limited understory vegetation cover, which may enhance soil erosion (Ribolzi et al., 2017).

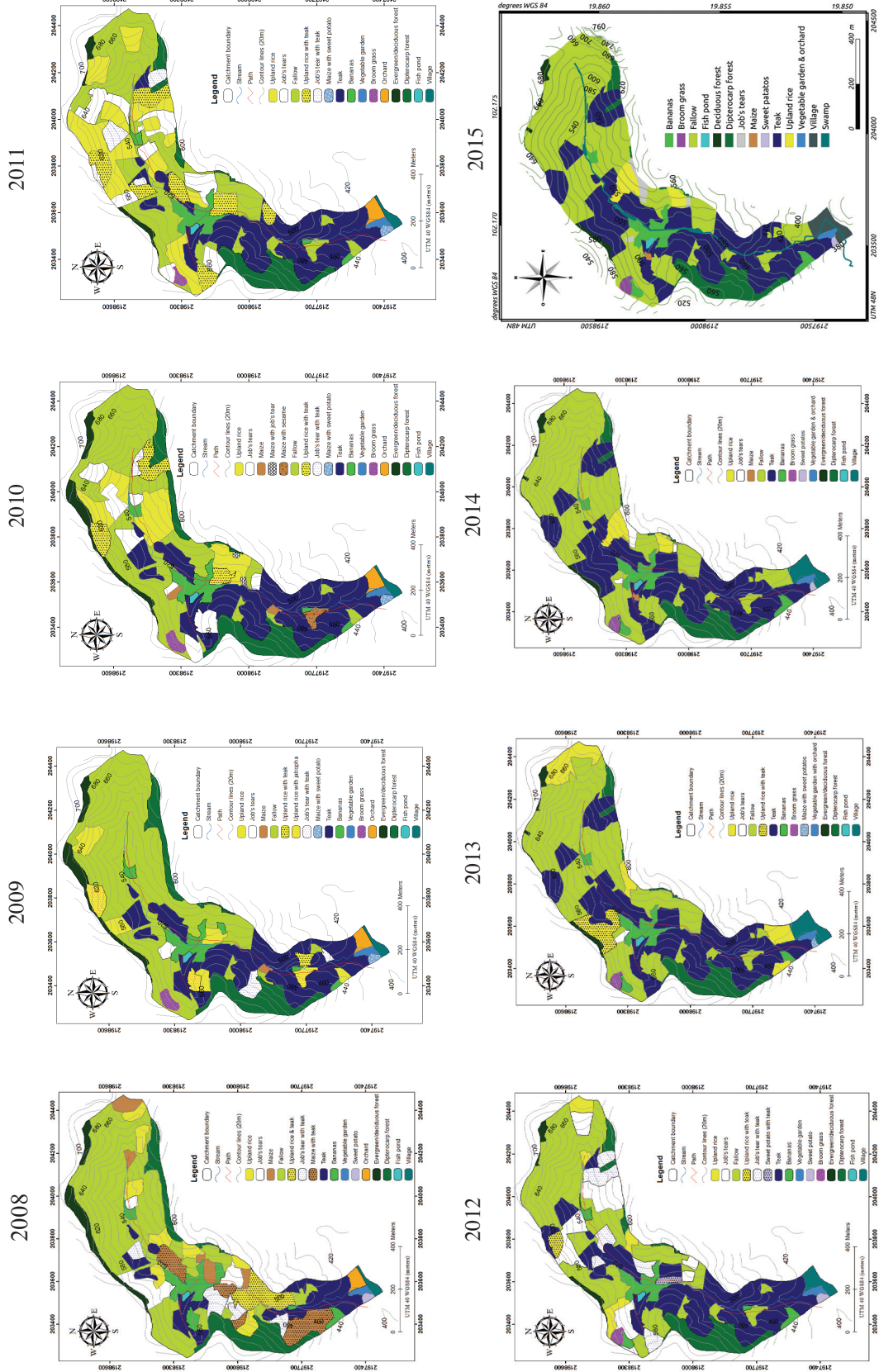


Fig 2.3: Land use changes from 2008 to 2015 in the Houay Pano catchment, Laos

2.2. Field work

2.2.1. Rain simulations and mesocosm/microcosm experiments

Rain simulations experiment

Experimental rain simulations were performed at two occasions during this thesis. The first rain simulations were conducted in the Dong Cao catchment on soils that had been amended with different organic fertilizers (compost, vermi-compost and biochar). The second rain simulation was conducted in the Houay Pano catchment under different LU including teak with / without understory and upland rice (Fig 2.4). The rain simulation was conducted according to the protocol detailed in Janeau et al. (2014). Briefly, two rain events were applied to each 1m² plot at a 24 h interval with a rain intensity of 90 mm h⁻¹ during 40 min (~585 J m⁻²). This intensity corresponds to an intense monsoonal rain event. Each 1m² plot was embedded approximately 10 cm into the soil surface and each frame was placed at between 4 to 6 m distances from the adjacent plots to ensure that there was no secondary influence from the other simulations. Between simulations, each plot was covered with PVC sheeting to avoid the influence of natural rainfall. Runoff water from the soil surface was drained from the bottom side of the plot via a drainage channel and was collected through a hole (Fig 2.4).



Fig 2.4: Rain simulation in the Houay Pano catchment, in the teak without understory (TWO) treatment

Mesocosm experiment

From the rain simulations conducted in Dong Cao catchment, runoff water was collected to perform mesocosm experiment (Fig 2.5). Ten liters of runoff water were collected from the soils amended with different organic fertilizers (compost, vermi-compost and biochar) and soil only control

was added into 100 L of lake water. The mixed water in mesocosms was incubated for 16 days. The mesocosms were daily mixed manually, and samples for measuring nutrient, DOC and Chlorophyll a concentration (days 0, 1, 2, 3, 5, 7 and 16) and bacterial diversity (day 0 and 16) were collected.

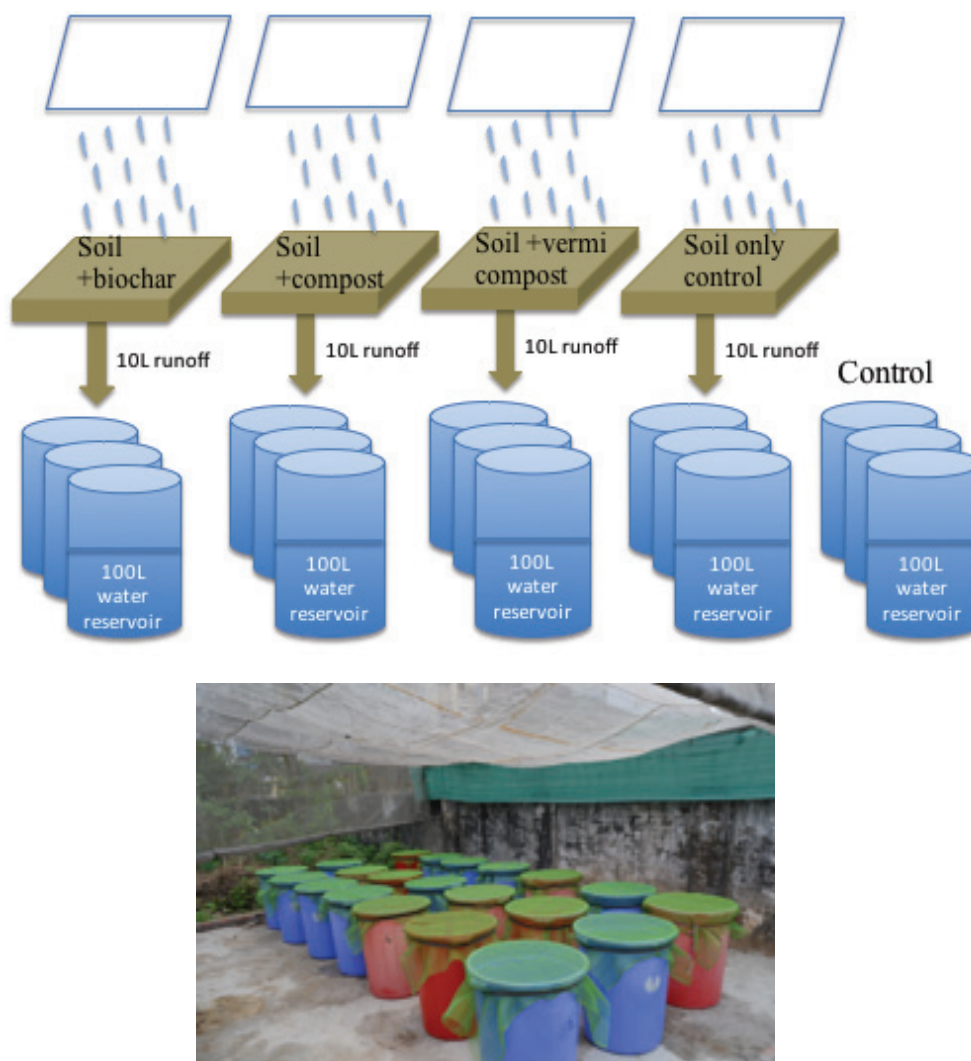


Fig 2.5: Mesocosm experiment in the Dong Cao catchment

Microcosm experiment

In a microcosm experiment, the overland flow was produced with soils under different LU including two-year and eight years fallow (2YF and 8YF) and teak with/without understory (TW/TWO). The different overland flows were added directly into stream water collected at the upper stream station (S4). The shift of bacterial community was assessed during 4 days incubation in the treated microcosms as well as in the control microcosm (stream water only) (Fig 2.6).

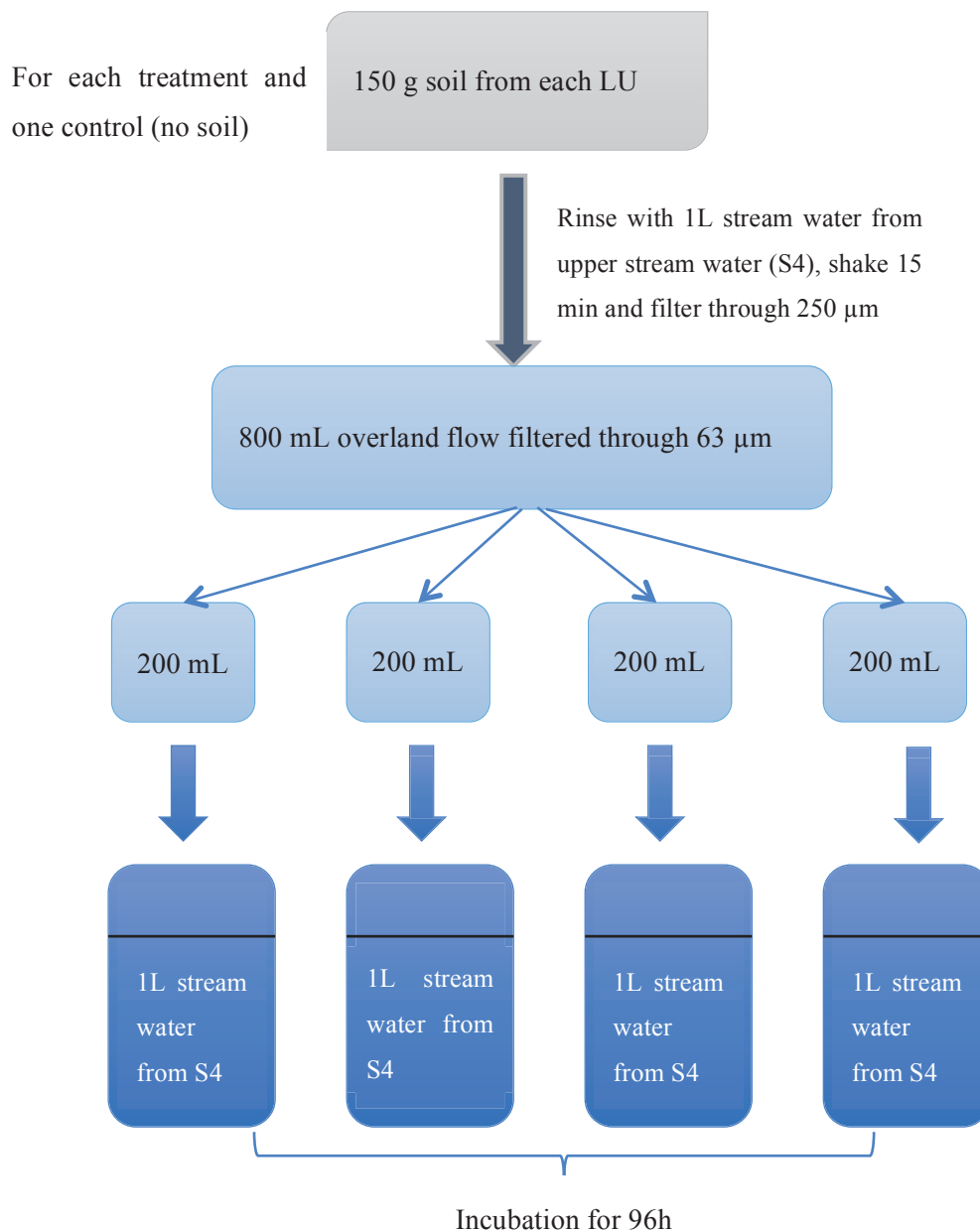


Fig 2.6: Microcosm experiment in the Houay Pano catchment

2.2.2. Sampling

Soil sampling

For the experiment of rain simulation, three soil cores (0-10 cm) were collected from each LU: teak with understory (TW), teak without understory (TWO) and upland rice (UR) from the Houay Pano catchment, Lao.

For the microcosm experiment, 20 soil cores (0-10 cm deep) along a 25m-transect were collected and pooled from each LU: teak with understory (TW) and teak without understory (TWO), 2 years fallow after 7 years rotating cultures (2YF), 8 years fallow after 7 years rotating cultures (8YF) from the Houay Pano catchment, Laos.

All soil cores were transported to the laboratory where they were then pooled and homogenized by 2-mm sieving and roots were removed.

Water sampling

Water samples were collected from the lake mesocosm incubated with runoff water under soil with different organic fertilizers from the rain simulation performed in the Dong Cao catchment and stream microcosm incubated with runoff water collected from the rain simulations performed in the parcels under different LU in the Houay Pano catchment.

Stream water in five sites along the stream was collected at base flow in during the wet season (June 12th, 2014) and dry season (March 22nd, 2015) (Fig 2.7). The sampling sites included:

- RIB72: Upmost site in the catchment, surrounded mostly by fallow and teak plantations
- S1: First gauging station on the stream, surrounded by bananas and teak plantations as well as rotating LU
- RIB48: Typical upland bog and surrounded with teak plantation
- S4: Second gauging station along the stream and surrounded with teak plantations
- S9: Outlet of the village Lak Sip

Water samples at different stations (S4, S8 and S10) were also collected during a time series of a flood event in the Houay Pano catchment (Fig 2.7). S4 is the second gauging station of the stream that is surrounded with teak plantations. Two additional stations (S7 and S8) draining two sub-basins (0.6 ha each) that are connected to the main stream between S1 and S4 were also monitored. Houay Xon River (22.4 km² catchment), of which the Houay Pano stream is a tributary, is continuously monitored at station S10 just downstream of the confluence with the Houay Pano catchment (Fig 2.7).

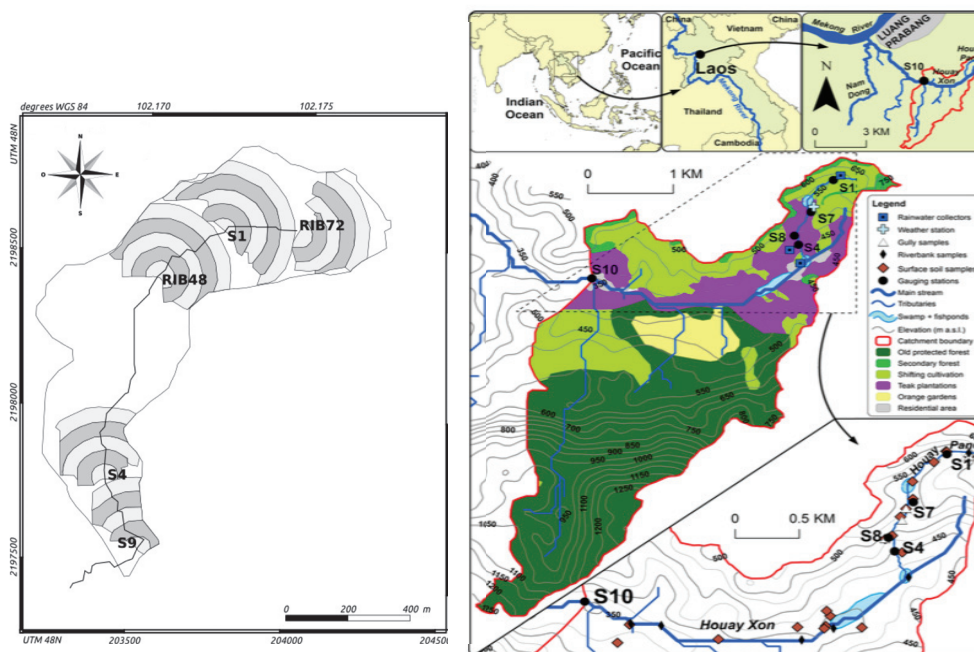


Fig 2.7: Sampling sites at base flow (left panel, Le *et al.* 2018) and during flood event (right panel, Evrard *et al.* (2016))

Samples used for DNA extraction

For the water samples from the mesocosm incubation in the Dong Cao catchment, 50 ml of sample were filtered onto separate 0.2 μm Supor filters.

For the water samples in the Houay Pano catchment, 50 ml were successively filtered through a 3 μm pore size filter for the Particle-attached (PA) fraction (3–20 μm) and a 0.22- μm polycarbonate filter for the Free-living (FL) fraction (0.22–3 μm).

All filters were kept at -20°C until DNA extraction.

Samples for DOC and colored dissolved organic carbon (CDOM)

All water samples were filtered immediately through Whatman GF/F glass fiber filters. For the determination of DOC concentration, duplicate 30 ml filtered samples were kept in pre-combusted (450°C, overnight) glass tubes, preserved with 36 μL 85% phosphoric acid (H_3PO_4) and sealed with a Teflon lined cap. Samples were stored at ambient temperature and in the dark until measurement.

For CDOM, 100-ml filtered samples were stored in pre-cleaned, 125-ml, amber glass bottles sealed with Teflon lined caps. After collection, the samples were stored frozen (-20°C) until measurement. Before measurement in the laboratory, the frozen samples were removed from the freezer and were allowed to slowly warm to room temperature, then were re-filtered at 0.2 μm (Sartorius Minisart NML Syringe filters) before measurement.

2.3. Laboratory work

2.3.1. Measurement of DOC, nutrients and Chlorophyll a

DOC concentration was measured on a Shimadzu TOC V_{CPH} analyzer following the method described in Rochelle-Newall et al. (2011). TN and TP concentrations were determined following the standard methods for the examination of water and wastewater (Clesceri et al., 1999). Concentrations of Chl a were determined fluorometrically from 30 mL subsamples filtered onto 25 mm Whatman GF/F filters according to Holm-Hansen et al. (1965). Total suspended sediment (TSS) samples were grounded with an agate mortar, weighed and packed into tin containers (5x9 mm). Total organic carbon (TOC) were measured using the Elementar VarioPyro cube analyzer on line with a Micromass Isoprime Isotope Ratio Mass Spectrometer (IRMS) following the method described in Huon et al. (2017).

2.3.2. Measurement of CDOM

Fluorescence was measured with a Turner Trilogy Fluorometer using the UV module insert with an excitation wavelength of 350 nm and emission wavelength of 410 - 450 nm. A milliQ water blank was subtracted from each measurement.

Absorption was measured on an Analytica Jena Specord 205 UV-VIS spectrophotometer from 200-750 nm using a 1 to 10 cm quartz cell, depending on the absorption of the samples with Milli-Q water as a blank. The absorbance values were converted to absorption coefficient, a (λ), m^{-1} (Green and Blough, 1994). The optical characteristics of CDOM measured in this thesis are presented in Table 2.1.

Excitation – Emission – Matrices (EEM) measurements were made on a Gilden Fluorosens fluorometer using a 1 cm quart cuvette with 5 nm bandwidths for excitation and emission at an integration time of 100 ms. Excitation scans were made over a range of 200 to 450 nm at 5 nm increments and emission from 220 to 600 nm at 2.5 nm increments. EEMs were corrected for inner filter effects and the manufacturers' machine correction was applied. EEM fluorescence of Milli-Q water blank was subtracted from that of the sample EEM, and corrected EEM was then converted to RU units. PARAFAC analysis was carried out in MATLAB (version R2016a 9.0.0) with the DrEEM toolbox for MATLAB (Murphy et al., 2013) in order to decompose the fluorescence signal into a series of tri-linear structures.

Table 2.1: Optical characteristic of dissolved organic carbon (DOC)

DOC quality index	Reference	Definition and significance
Specific UV absorbance (SUVA ₂₅₄)	Weishaar et al. (2003)	UV absorbance at 254 nm divided by DOC concentration in mg CL ⁻¹ , is a proxy for the degree of aromaticity of DOM. High values of SUVA indicate higher aromaticity
Slope ratio (Sr)	Helms et al. (2008)	Ratio the slope of the shorter UV wavelength region (275-295 nm) to that of the longer UV wavelength region (350-400nm) can be used as a proxy for molecular weight (MW). Sr decreases with increasing MW.
Humification index (HIX)	Ohno (2002)	HIX was calculated from excitation 255 nm as the ratio of the peak area under each curve at emission 434-480nm and 300-346 nm Indicates humic content of DOM, ranges from 0 to 1 and increases with increasing degree of the humification
Fluorescence index (FI)	McKnight et al. (2001), Cory and McKnight (2005)	Ratio of fluorescence emission intensities at wavelength region of 470:520 nm excited at 370 nm. FI values around 1.3 suggest a dominant terrestrial, higher-plant DOM source and values around 1.8 suggest a predominant microbial DOM source

2.3.3. Measurement of metabolic capacity

Bacterial metabolic capacity was measured using Biolog[®] Ecoplate for all experiments, in the Houay Pano catchment, except during the survey of the flood event. These 96-wells microplates include 31 different carbon-based substrates and a water control in triplicate. Each well also contains growth media and a tetrazolium violet dye that turns purple when the substrate is oxidized. Any color development indicates utilization of carbon sources inherent in the sample (Tiquia et al., 2008). For each sample, 150 µl were inoculated into each well and the plates were then incubated at 25 - 27°C for 96 h. Well color development at 590 nm was measured using an iMark Microplate Reader (BioRad) every

24h. Utilization of each group of substrates was measured for 96 h after subtracting the appropriate water blank.

2.3.4. DNA extraction

DNA extraction from water samples was conducted following a modified protocol adapted from Fuhrman et al. (1988) DNA extraction followed the procedure described below (Fig 2.8):

- Bacterial cell wall was broken by both chemical processes (lysis buffer, lysozyme) and mechanical processes (cycles of freeze-thaw and shaking with glass beads)
- Sodium dodecyl sulfate (SDS 10%) and proteinase K were used to remove lipid membrane
- A sodium chloride (NaCl) and a cetyltrimethylammonium bromide (CTAB) solution were added to separate DNA from protein
- Nucleic acids were then extracted twice from digestion products with phenol-chloroform-isoamyl alcohol (25:24:1)
- Nucleic acid was precipitated by adding isopropanol

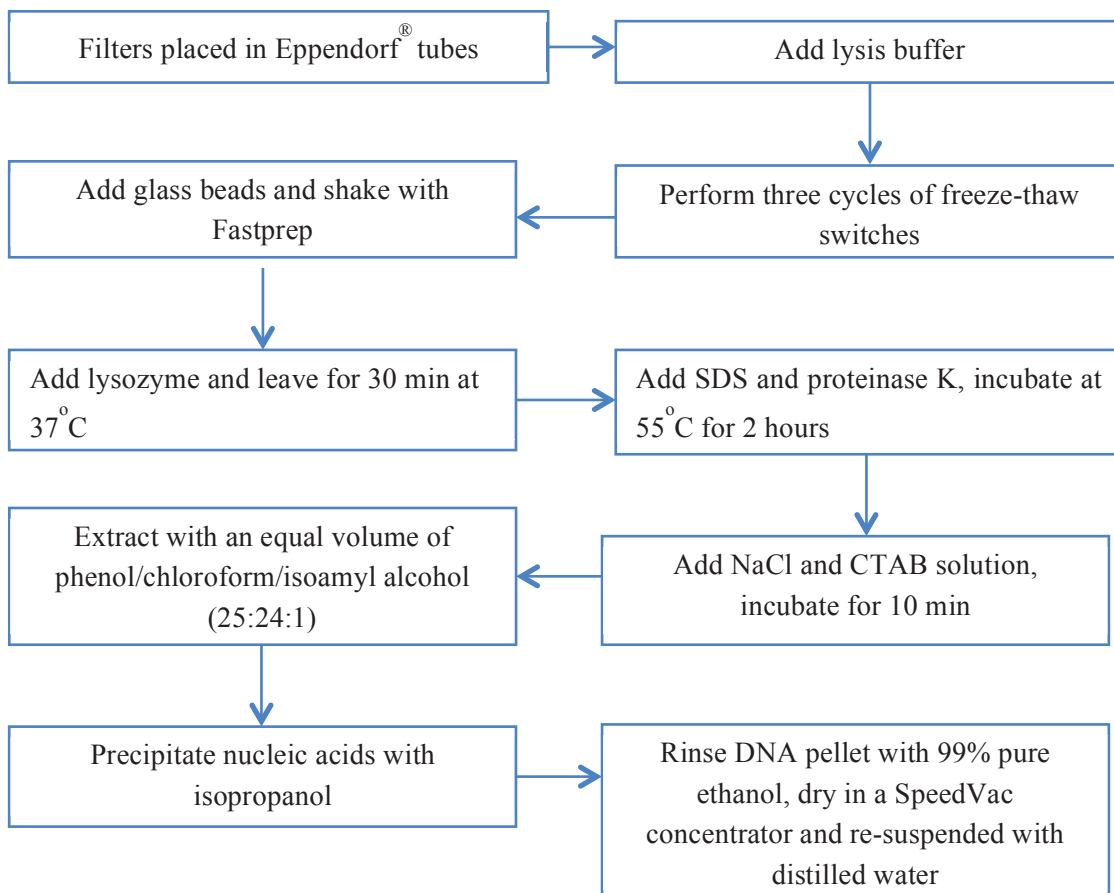


Fig 2.8: DNA extraction procedure for water samples

After extraction, the DNA products were sent to Molecular Research DNA (Shallowater, Texas, USA) for PCR amplification and high throughput sequencing using Illumina MiSeq (2x 250 bp). The PCR primers 515F (5'-AGRGTTTGATCMTGGCTCAG-3') and 806R (5'-GTNTTACNGCGGCKGCTG-3') (producing a 291bp amplicons) (Capone et al., 2011) with sample-specific barcodes on the forward primer were used to amplify the V4 variable region of the 16S rRNA gene. Pooled PCR products were purified using calibrated Agencourt AMPure XP magnetic beads according to the manufacturer's guidelines. The pooled and purified PCR product was then used to prepare a DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing was performed at MR DNA (www.mrdnlab.com, Shallowater, TX, USA) on an Illumina MiSeq machine following the manufacturer's guidelines.

2.4. Sequence processing and data analysis

The MOTHUR software v. 1.33 was used to process 16S rRNA gene sequence reads (Schloss et al., 2009). Short reads (<250 bp) and reads with ambiguous primer or barcode sequences were discarded. Sequencing errors were reduced by aligning the remaining reads to the SILVA database, screening the alignment to the overlapping region, and pre-clustering sequences distant by < 2bp. Chimeric sequences were identified using the integrated version of UChime and removed accordingly. To avoid misinterpretation, sequences that were classified as "Chloroplast", "Mitochondria", or "unknown" lineages were removed before clustering into Operational taxonomic units (OTUs). Sequences with a pairwise distance <0.03 substitutions per nucleotide were clustered in OTU and considered for further analyses.

Alpha and Beta diversity analyses were all performed using MOTHUR v. 1.33 (Schloss et al., 2009). Alpha diversity analysis was performed on the whole data set with a normalized sequence to the lowest read number in the data set. The Chao1, Invsimpson and Shannon indices, all common measures of diversity within a sample (alpha diversity), were used to describe species richness and evenness, respectively.

Chao 1 index was measured as:

$$S_{\text{Chao 1}} = S_{\text{obs}} + f_1(f_1 - 1) / 2(f_2 + 1)$$

where S_{obs} is the observed number of OTU, f_1 the number of OTUs with only one sequence (singleton) and f_2 , the number of OTUs with two sequences (doubleton).

The **invsimpson** calculator is the inverse of the classical Simpson diversity estimator:

$$D = 1 / \sum p_i^2$$

where D is the Simpson index, $p_i = n/N$ and n, the individual of one particular species and N the total number of individual.

Shannon index was calculated following the equation below:

$$H = - \sum p_i \ln p_i$$

where $p_i = n/N$ and n , the individual of one particular species and N the total number of individual

Beta diversity was analyzed with a non-metric multi-dimension scaling (NMDS) based on Bray-Curtis dissimilarity to estimate the dissimilarity in structure between all samples using the *metaMDS* and *ordisurf* functions from the *vegan* package in R studio (Oksanen et al., 2017).

Venn diagrams were built to show shared species between soil samples, runoff water under different LU, and between sites along the stream in MOTHR software (v. 1.33).

Taxonomic assignments were performed on the alignment of consensus sequences with the RDP database (Cole et al., 2005) using MOTHR v. 1.33 (Schloss et al., 2009).

2.5. Statistical analyses

To test the significance of the differences between treatments (Mesocosm experiment) and DOC and optical characteristics between different samples (*In situ* investigation), Analysis of Variance (ANOVA) was used after checking that the assumptions of the ANOVA were met. When necessary the data was log-transformed to assure normality. When transformation did not assure normality, a non-parametric Kruskal–Wallis test was used.

To measure the correlations between DOC concentrations and optical index, and alpha diversity (*i.e.* Chao, Invsimpson, Shannon index), Pearson correlation coefficient with the *Hmisc* package in R (Harrell, 2018) was calculated.

Canonical Correspondence Analysis (CCA) with the *VEGAN* package in R (Oksanen et al., 2017) was used to analyze the relationships between DOC/CDOM and bacterial community structure.

ANOSIM test was performed with Bray-Curtis dissimilarity using the *VEGAN* package in R to determine the significance of the difference in bacterial community structure. The *env.fit* function was applied to the Bray-Curtis dissimilarity matrices (*VEGAN* package in R) (Oksanen et al., 2017) to evaluate the significance of relationship between PA and FL bacterial structure and the environment factors.

Partial Least Square (PLS) analysis was performed to assess the effect of LU and its legacy on bacterial diversity. All factors computing between the bacterial diversity and the environment tables were conducted using the R packages *PLSDEPOT* (Sanchez, 2012) and the graphical identification of the relevant variables was performed using *ADE-4* (Thioulouse et al., 1997).

All differences were considered significant when the p-value of the tests was lower than to 0.05

CHAPTER 3

EFFECTS OF DIFFERENT RUNOFF ON AQUATIC BACTERIAL DIVERSITY AND FUNCTIONING AT MESOCOSM SCALES

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Abstract

Organic fertilizer application is often touted as an economical and effective method to increase soil fertility. However, this amendment may increase dissolved organic carbon (DOC) runoff into downstream aquatic ecosystems and may consequently alter aquatic microbial community. We focused on understanding the effects of DOC runoff from soils amended with compost, vermicompost or biochar on the aquatic microbial community of a tropical reservoir. Runoff collected from a series of rainfall simulations on soils amended with different organic fertilizers was incubated for 16 days in a series of 200 liters mesocosms filled with water from a downstream reservoir. We applied 454 high throughput pyrosequencing for bacterial 16S rRNA genes to analyze microbial communities. After 16 days of incubation, the richness and evenness of the microbial community present decreased in the mesocosms amended with any organic fertilizers, except for the evenness in the mesocosms amended with compost runoff. In contrast, they increased in the reservoir water control and soil-only amended mesocosms. Community structure was mainly affected by pH and DOC concentration. Compared to the autochthonous organic carbon produced during primary production, the addition of allochthonous DOC from these organic amendments seemed to exert a stronger effect on the communities over the period of incubation. While the *Proteobacteria* and *Actinobacteria* classes were positively associated with higher DOC concentration, the number of sequences representing key bacterial groups differed between mesocosms particularly between the biochar runoff addition and the compost or vermi-compost runoff additions. The genera of *Propionibacterium spp.* and *Methylobacterium spp.* were highly abundant in the compost runoff additions suggesting that they may represent sentinel species of complex organic carbon inputs. Overall, this work further underlines the importance of studying the off-site impacts of organic fertilizers as their impact on downstream aquatic systems is not negligible.

Keywords: DOC, compost, biochar, aquatic microbial community, mesocosms

3.1. Introduction

Organic fertilizers are cited as being a sustainable option for improving soil quality and crop yields in degraded soils (Eghball, 2002). Many studies have shown that the incorporation of compost or vermi-compost into soil improves water retention, cation exchange capacity, soil structure, organic carbon content and nutrient quality (Rivero et al., 2004). Compost and vermi-compost are formed by bio-oxidation and stabilization of vegetable and animal waste by microorganisms in the case of compost and through the joint action of earthworms and microorganisms in the case of vermi-compost (Vivas et al., 2009). Biochar, which is formed by the pyrolysis of organic matter such as bamboo or rice straw, is also used as a soil amendment and it is particularly promoted as a low cost option in tropical countries. As with the other organic amendments, biochar has been shown to increase soil moisture retention, improve soil structure, and reduce nutrient leaching (Anderson et al., 2011; Doan, 2015) as well as reducing greenhouse gas emissions from soils (Lehmann, 2007).

Although the positive impacts of organic amendments on soil quality and crop yield in both temperate and tropical countries have been amply demonstrated, the impact of these amendments on soil runoff and the impact of that runoff on adjacent aquatic systems has been less studied. The concentration of DOC in soil runoff is strongly influenced by soil organic carbon content, soil erosion, vegetation cover, amounts and intensities of rainfall, as well as type of composting applied (Mailapalli et al., 2012; Worrall et al., 2012; Janeau et al., 2014; Pommier et al., 2014). It is therefore important to understand the fate of this DOC, as runoff has the potential to impact adjacent terrestrial and/or aquatic ecosystems (Jacinthe et al., 2004). Moreover, the impacts of agricultural runoff *e.g.* nitrogen fertilizers, on adjacent aquatic systems have been the focus of many studies in temperate and developed countries for many years much less is known about the impacts of agricultural practices in the tropics. In addition, although many studies have examined the impacts of inorganic fertilizers (Howarth, 1998; Howarth et al., 2012) or pesticides and herbicides (Wohlfahrt et al., 2010; Dalton et al., 2015), less have focused on the impacts of organic fertilizers on downstream aquatic ecosystems. This is despite the known importance of organic matter for microbial processes, aquatic biogeochemistry and microbial community structure.

The changes in microbial composition and metabolic activity are probably tightly linked to shifts in the quantity and quality of DOM (Fang et al., 2014). Although the influence of changing DOM on bacterial diversity has been investigated in cultures (*e.g.* Sarmiento et al., 2013), microcosms (Pommier et al., 2014) and coastal and oceanic waters (*e.g.* Cottrell and Kirchman, 2000; Gómez-Consarnau et al., 2012), a comprehensive understanding of how aquatic microbial communities respond to shifts in DOC bioavailability in runoff from agricultural soils in tropical environments is, to our knowledge, still lacking. Therefore, in this study, we investigated the impact of runoff from compost, vermi-compost and biochar on reservoir bacterial communities in mesocosm experiment. The objectives of this study were to determine how runoff from compost, vermi-compost and biochar influences aquatic bacterial

communities in a typical tropical headwater reservoir. We hypothesized 1) that the application of organic fertilizer (compost, vermi-compost, biochar) would lead to an increase in the concentration of DOC in runoff from agricultural soils and into the adjacent aquatic systems; 2) that the addition of allochthonous DOC into a downstream water body would alter bacterial community structure, inducing the growth of key taxonomic groups that were adapted to different sources of DOC; and 3) that biochar runoff, considering its different chemical structure, would induce different bacterial groups compared to compost and vermi-compost runoff additions.

3.2. Methods

Study area

The rain simulation experiments were carried out in the peri-urban Dong Cao catchment, located in hills of Tien Xuan in the Thach That district of Ha Noi, Vietnam (20° 57' 30" N, 105° 29' 15" E; elevation 300 m a.s.l). The catchment has an area of 50 ha and is bisected by a small stream which is connected to a downstream reservoir (volume of 700 000 m³).

The dominant soil type in this catchment is an Acrisol, i.e. tropical clay-rich Ultisol (FAO, 1998; Soil Survey Staff, 1999). The climate is a monsoon climate with the main precipitation falling within the summer from April to October. The twelve year (1999 - 2011) mean annual precipitation measured in this sub-catchment was 1502 mm y⁻¹ with a minimum of 1262 mm y⁻¹ in 2010 and a maximum of 2506 mm y⁻¹ in 2001 (Podwojewski et al., 2008; Tran et al., 2011).

Rainfall simulations

The rainfall simulation took place in April 2012 at the end of the dry season. We examined in triplicate runoff and organic matter export under three types of amendment plus a soil only control (Table 3.1). The different amendments correspond to three different options that are promoted as improving soil organic carbon content and fertility: compost, vermi-compost and biochar.

Table 3.1: The different treatments applied to the plots and the average Dissolved organic carbon (DOC) concentration (mean +/- se) in runoff from that treatment. Different letters indicate a significant difference in DOC, total nitrogen (TN) or total phosphorous (TP) concentration. Each treatment was tested in triplicate

Practice	Addition	DOC (mg L ⁻¹ C)	TN (mg L ⁻¹ N)	TP (mg L ⁻¹ P)
Soil only	No addition	2.1 (0.3) ^c	1.6 (1.0) ^a	0.05 (0.02) ^a
+ Compost	2 kg of compost	8.1 (2.3) ^a	1.5 (0.5) ^a	0.7 (0.6) ^b
+ Vermicompost	2 kg of vermicompost	3.8 (0.9) ^{b,d}	1.8 (0.9) ^a	1.4 (0.3) ^b
+ Biochar	340 g of biochar	2.6 (0.4) ^{c,d}	1.2 (0.5) ^a	0.08 (0.05) ^a

Rain simulations were conducted as in Janeau et al. (2014). Runoff water from the soil surface was drained from the bottom side of the plot via a drainage channel and was collected through a hole. Each plot was embedded approximately 10 cm into the soil surface and each frame was placed at between 4 to 6 m distances from the adjacent plots to ensure that there was no secondary influence from the other rain simulations. The plots were set out along a contour line in a field that is regularly used for crop cultivation. The slope of the plots varied between 28 and 45 % with an average slope of $36.1 \pm 8.5\%$.

For each simulation, two successive rain events were applied at a 24 hours interval with a rain intensity of 90 mm h^{-1} during 40 min ($\sim 585 \text{ J m}^{-2}$). This intensity corresponds to an intense monsoonal rain event. Each plot was covered with PVC sheeting to avoid the influence of natural rainfalls occurring between the simulations.

Runoff water from the 1 m^2 plot was collected in a large, clean bucket during each rain event. At the end of each of the two simulations per plot, 5 L of runoff water was collected and stored at $4 \text{ }^\circ\text{C}$ for use in the aquatic mesocosm incubation (see below). Total nitrogen (TN), total phosphorus (TP) and DOC concentrations were measured at the end of each rain simulation.

Aquatic mesocosm incubation

In order to assess the impact of runoff water from different amendments on aquatic microbial communities, a short, 16 days mesocosm incubation was conducted immediately after the rain simulations. Fifteen plastic containers of 200 L volume corresponding to the 3 replicates of the 3 organic addition treatments plus the 3 soil only controls and 3 replicate reservoir water controls were filled with 100 L of water collected from the reservoir in the studied catchment.

Ten liters of runoff, 5 L from each of the two simulations, was added at the beginning of the incubation to the respective mesocosms, except for the reservoir controls. These additions were calculated based on the data of Bui Tan Yen et al. (2014) on runoff of water from the watershed to the reservoir and on annual precipitation volumes. Annual rainfalls in 2012 and 2013 in the Dong Cao watershed accounted for 1 200 and 1 800 mm, respectively, of which between 80 and 90 % occurred over the 5-month period between May and September. While the mean peak flow of water runoff to the lake is estimated around $0.11 \text{ m}^3 \text{ s}^{-1}$ (i.e. $10\,000 \text{ m}^3 \text{ d}^{-1}$; Bui Tan Yen et al., 2014), the volume of the reservoir was estimated to seasonally change between $640\,000 \text{ m}^3$ and $1\,120\,000 \text{ m}^3$ (Trinh et al., 2016). In other words, over the 5-month rainy season the water volume increases by approximately 43 % ($480\,000 \text{ m}^3$), the majority of which comes from runoff. Compared to natural conditions, the chosen 10 L-addition of runoff water to 100 L reservoir water therefore represents a typical addition occurring during one month of the rainy season.

Each mesocosm was covered with one layer of mosquito screening to protect from falling debris such as leaves and the incubation was conducted outside and ran for 16 days. Mesocosms were mixed manually daily and samples for nutrients, DOC and Chlorophyll *a* (Chl*a*) concentration (Days 0, 1, 2, 3, 5, 7, 16) and bacterial diversity ($T_i = \text{Day } 0$ and $T_f = \text{Day } 16$) were collected. Temperature, dissolved oxygen (DO) and total dissolved solids (TDS) were measured daily with a Hydrolab 5 multiprobe in each mesocosm after mixing.

Nutrients and DOC

For the determination of DOC concentration, duplicate 30 mL samples were filtered (Whatman GF/F), collected in pre-combusted ($450 \text{ }^\circ\text{C}$, overnight) glass tubes, preserved with $36 \text{ } \mu\text{L}$ 85 % phosphoric acid (H_3PO_4) and sealed with a Teflon lined cap. Samples were stored at ambient temperature and in the dark until measurement. DOC concentration was measured on a Shimadzu TOC V_{CPH} analyzer following the method described in Rochelle-Newall et al. (2011).

TN and TP concentrations were determined following the standard methods for the examination of water and wastewater (Clesceri et al., 1999). TN was measured on unfiltered samples using the Total Kjeldahl Nitrogen (TKN) method (4500-Norg B) (Clesceri et al., 1999). TP was determined on unfiltered samples after addition of concentrated HClO_4 solution and heating at $150 \text{ }^\circ\text{C}$ for 2 h followed by determination of PO_4 colorimetrically at 880 nm with the ascorbic acid method (Standard method 4500-P E (Clesceri et al., 1999). All colorimetric measurements were conducted on a GBC Cintra 40 spectrophotometer (Australia). Concentrations of Chl*a* were determined fluorometrically from 30 mL subsamples filtered onto 25 mm Whatman GF/F filters according to Holm-Hansen et al. (1965). The rainwater used for the simulations was taken from a nearby spring and the concentrations of DOC (0.74 mg C L^{-1}), TN (0.024 mg L^{-1}) and TP (0.025 mg L^{-1}) were subtracted from the DOC, TN and TP runoff measurements.

DNA extraction and 16S rRNA gene sequencing

Samples of 50 mL from each incubation were filtered onto separate 0.2 μm ($\phi = 47$ mm) Whatman Nuclepore polycarbonate membranes at the initial (T_i) and final time (T_f) of the incubation. DNA was extracted using an in-house standard protocol. Filters were thawed and lysozyme (1 mg mL⁻¹ final) was added and left for 30 min at 37 °C. Sodium dodecyl sulphate (SDS, 1% final) and proteinase K (100 μg mL⁻¹ final) were added and incubated overnight at 55 °C. Cell lysates were extracted with an equal volume of phenol/chloroform/isoamyl alcohol [25 : 24 : 1] mixed thoroughly and centrifuged for 5 min (20 000 g). The upper phase of the centrifugation products were transferred to new tubes and an equal volume of chloroform/isoamylalcohol [24 : 1] was added and centrifuged for 5 min (20 000 g). The upper phase of the centrifugation products were transferred to new tubes and roughly 1/10 volume equivalent of sodium acetate (NaAc) [3M, pH = 5.2] was added together with 0.6 volume equivalent of isopropanol and incubated 1 h at -20 °C. A new centrifugation was performed at 20 000 g for 20 min at 4 °C. The pellet was washed with ice cold 70 % ethanol, dried in a SpeedVac concentrator® (Savant), re-suspended in 50 μL TE buffer, and quantified using the PicoGreen® fluometric quantification kit (molecular Probes). PCR amplification of the hypervariable regions V1-V3 of the 16S rRNA gene was performed using the primers 27F (5'-GAGTTTGATCMTGGCTCAG-3') and 518R (5'-WTTACCGCGGCTGCTGG-3'). Prior to pyrosequencing all PCR amplicons were pooled to equimolar ratio. The forward and reverse primers included both a 10 bp multiplex identifier (MID) in order to multiplex the samples during sequencing. Amplifications were performed in triplicate using the AmpliTaq Gold® 360 master mix (Applied Biosystems), according to the protocol of the manufacturer. Cycling conditions were as follows: an initial activation/denaturation step at 95 °C for 10 min; followed by 25 cycles of 95 °C for 40 s, 55 °C for 40 s, and 72 °C for 1 min; and a final 7-min extension at 72 °C. PCR products were then purified using the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) after excision of the amplicon from an agarose gel. Concentration of DNA of each identified PCR product was determined using Picogreen quantification and then PCR triplicates of a same site were pooled into equimolar concentrations. Pyrosequencing was then performed on a 454 GS-FLX Titanium (454 Life Sciences) by Macrogen, Korea. Data are publicly available at the National Centre for Biotechnology Information (NCBI) under the reference project ID *SUB1440290*.

Sequences processing and data analysis

The MOTHUR software (v. 1.33; Schloss et al., 2009) was used to process 16S rRNA gene sequence reads. Short reads (<250 bp) and reads with ambiguous primer or barcode sequences were discarded. Sequencing errors were reduced by aligning remaining reads to the SILVA database (Pruesse et al., 2007), screening the alignment to the overlapping region, and pre-clustering sequences distant by < 2bp. Chimeric sequences were identified using the integrated version of UChime (Edgar et al., 2011) and removed accordingly. To avoid misinterpretation, sequences that were classified as "Chloroplast", "Mitochondria", or "unknown" lineages were removed before clustering into Operational taxonomic

units (OTUs). Sequences with a pairwise distance < 0.03 substitutions per nucleotide were clustered in OTU and considered for further analyses. The estimated richness and evenness were calculated through Chao and invSimpson indices, respectively. Taxonomic assignments were performed on the alignment of consensus sequences with the RDP database (Cole et al., 2005).

To assess the relative influence of environmental parameters and changes in community structure, a canonical correspondence analysis (CCA) was performed between key environmental parameters (pH, TDS, DO and DOC) and community composition using R with the ‘vegan’ package (Dixon, 2003). A heatmap was performed to show the comparison and cluster analysis of microbial composition in the pool samples at the genus level using R with the “pheatmap” package (Kolde, 2012).

Statistical tests

Significant differences between the treatments were tested using the Xlstat 2012 (Addinsoft) software. Analysis of variance (ANOVA) was used to test the significance of the differences between treatments after checking that the assumptions of the ANOVA were met. When necessary, the data were log-transformed to assure normality. When a significant difference was observed, an *a posteriori* test (Fisher’s LSD) was used to distinguish groups. When transformation did not assure normality, a non-parametric Kruskal-Wallis test was used.

Metastats was used to test the difference of abundance to identify significantly abundant genera in the samples. Analysis of Similarity (ANOSIM) was used to test the effect of time on community structure in all samples.

3.3. Results

DOC in runoff

There were significant differences in the amount of DOC lost as runoff during the rain simulation between amendments ($p < 0.05$; Table 3.1). Compared to the soil only treatment (2.1 +/- 0.3 mg L⁻¹ C), DOC was over three times higher in the runoff from plots with compost (8.1 +/- 2.3mg L⁻¹ C). TN and TP also varied between treatments with the concentrations being lowest in the soil only runoff for TN and in the soil only and biochar runoff for TP.

Environmental variables of aquatic mesocosms

The temporal variation of the measured environmental parameters (temperature, DO, pH and TDS) was rather similar amongst treatments (Fig 3.1). Average temperature varied between 26 and 31°C over the course of the experiment (Fig 3.1a). DO concentration decreased from around 7.5 at day 0 to about 4 at day 12, thereafter it slightly increased to reach 5.5 by the end of the experiment (Fig 3.1b). pH varied between 7.8 and about 9 in all samples, decreasing from around 9 at day 0 to around 8 by days 11 - 13, it increased again to almost 9 on the last day of incubation (Fig 3.1c). TDS in the compost treatment was slightly higher than other treatments in all days of incubation. The TDS concentration gradually rose

and was highest on day 11 (0.09 g L^{-1}), thereafter it decreased to 0.07 g L^{-1} by the end of the experiment (Fig 3.1d).

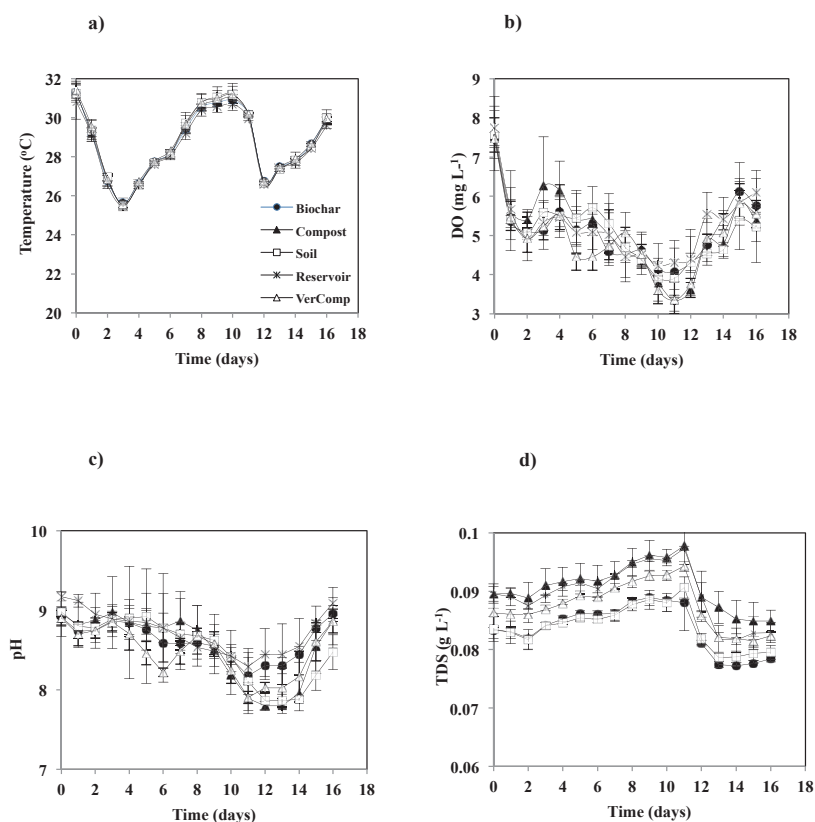


Fig 3.1: Environmental parameters during 16 days incubation. a) Temperature ($^{\circ}\text{C}$); b) dissolved oxygen (DO, mg L^{-1}); c) pH; d) total dissolved solids (TDS, mg L^{-1}). The mean and standard deviation are given for each treatment ($n=3$).

DOC and Chla

The addition of runoff to the reservoir water resulted in an increase in initial DOC concentration, relative to the reservoir (Fig 3.2a). This was particularly noticeable in the compost addition where initial concentrations of $3.7 \pm 0.3 \text{ mg C L}^{-1}$ were observed. This was in contrast to an initial reservoir DOC concentration of $3.4 \pm 0.1 \text{ mg C L}^{-1}$. The other additions resulted in initial DOC concentrations that varied between $3.1 \pm 0.2 \text{ mg C L}^{-1}$ for the soil only runoff experiment and $3.4 \pm 0.01 \text{ mg C L}^{-1}$ for the vermi-compost runoff addition. DOC concentration thereafter increased in all of the incubations. However, the rate of increase in DOC concentration was significantly higher in the compost addition ($p < 0.01$). Chla was highly variable during the experiment with no significant differences between treatments (Fig 3.2b). In general, Chla increased during the first few days to each a maximum on days 2-3 ($46.2 \pm 15 \mu\text{g L}^{-1}$) in the runoff addition mesocosms and day 5 ($43.2 \pm 21 \mu\text{g L}^{-1}$) in the reservoir water, and thereafter decreased towards the end of the experiment in all treatments ($13.7 \pm 6 \mu\text{g L}^{-1}$).

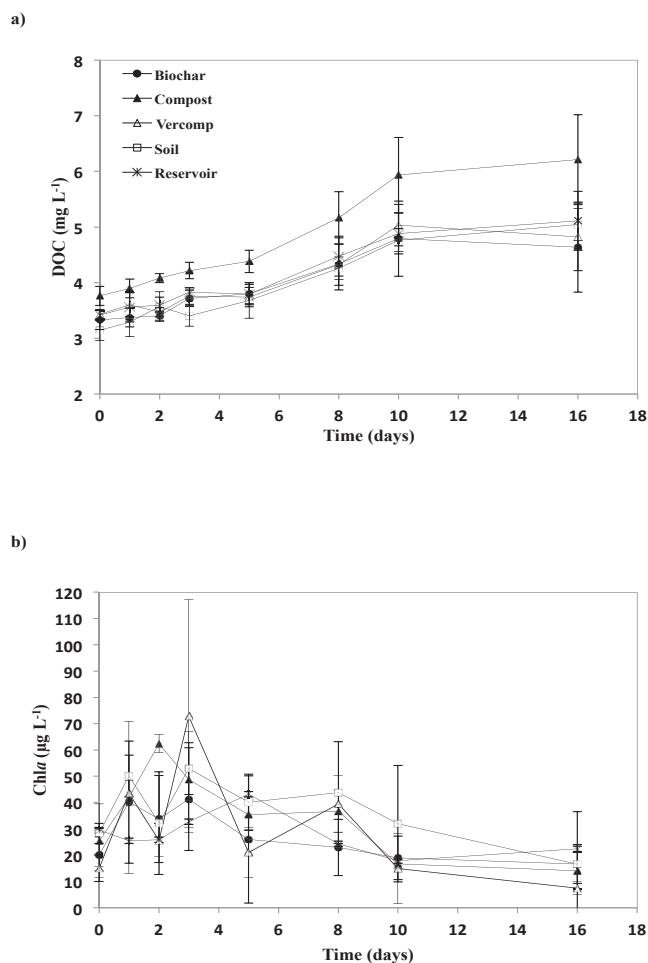


Fig 3.2: a) Concentration of DOC (mg L^{-1}) and b) Chla ($\mu\text{g L}^{-1}$) for each treatment. The mean and standard deviation are given for each treatment ($n=3$).

Estimated richness, evenness and structure of bacterial community

In total 327 327 sequences were obtained for all samples. At T_i , the estimated richness was highest in the mesocosms receiving runoff water from soil fertilized with biochar. In these mesocosms, the richness strongly declined by 55.7 % while it decreased by 46 % in the compost addition and by 35.6 % in the vermi-compost addition between the two measured time points (Fig 3.3a). In contrast, richness did not vary in the control mesocosms that had received runoff water from soil only or those that were only filled with reservoir water. Correspondingly, evenness was negatively impacted in the mesocosms with biochar and vermicompost runoff additions between T_i and T_f . However, evenness increased by 44 % in the control mesocosms that had received runoff from soil only and by 34 % in the reservoir water controls. It also slightly increased in the mesocosms receiving runoff water from soil amended with compost by 15% but it is highly variable between these mesocosms (Fig 3.3b).

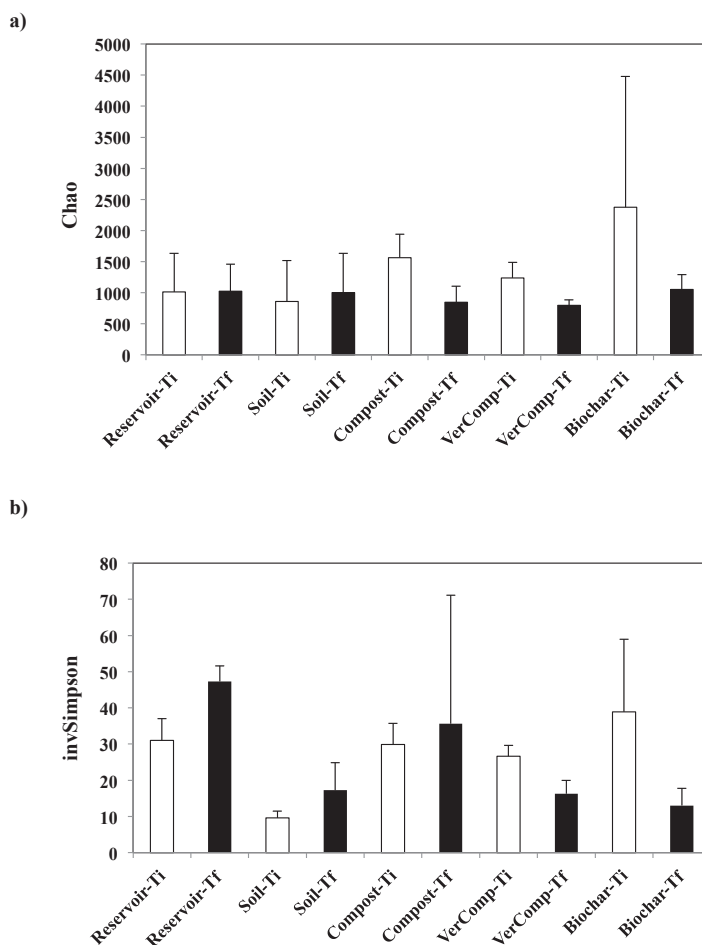


Fig 3.3: Estimated richness and evenness. (a) Chao index and (b) InvSimpson at Ti and Tf for all treatments. The mean and standard deviation are given for each treatment ($n=3$).

Also, community structure significantly changed through time ($P < 0.01$). The canonical correspondence analysis (CCA) between key environmental parameters (pH, TDS, DO and DOC) and community composition showed that the proportion of the total variance explained by all environmental factors was 22.76 %. The variance explained by the first three constrained axes (CCA1, CCA2, CCA3) were 18 %, 7.6 % and 3.3 % respectively (Fig 3.4). CCA constructed at the taxonomic level of class indicated specific effects of environmental parameters on microbial community structure: DOC and pH were two important factors affecting microbial community ((Fig 3.4). DOC was not significantly correlated with DO ($r = 0.23$, $P = 0.2759$), but was negatively correlated with TDS ($r = -0.4$, $P = 0.05$).

In general, all samples at T_f were correlated with high DOC and TDS. The occurrence of species belonging to the classes of *Alphaproteobacteria*, *Gammaproteobacteria*, *Betaproteobacteria*, *Actinobacteria* and *Armatimonadia* were positively correlated with higher DOC concentration at T_f . Moreover, the community structure for each treatment at T_i was clearly separated from those at T_f , except for the soil only treatment (Fig 3.4). Surprisingly, two samples from this treatment were very

different from all others at both T_i and T_f , suggesting that it may be affected by runoff collection or may be have been contaminated during the filling of the incubation tanks.

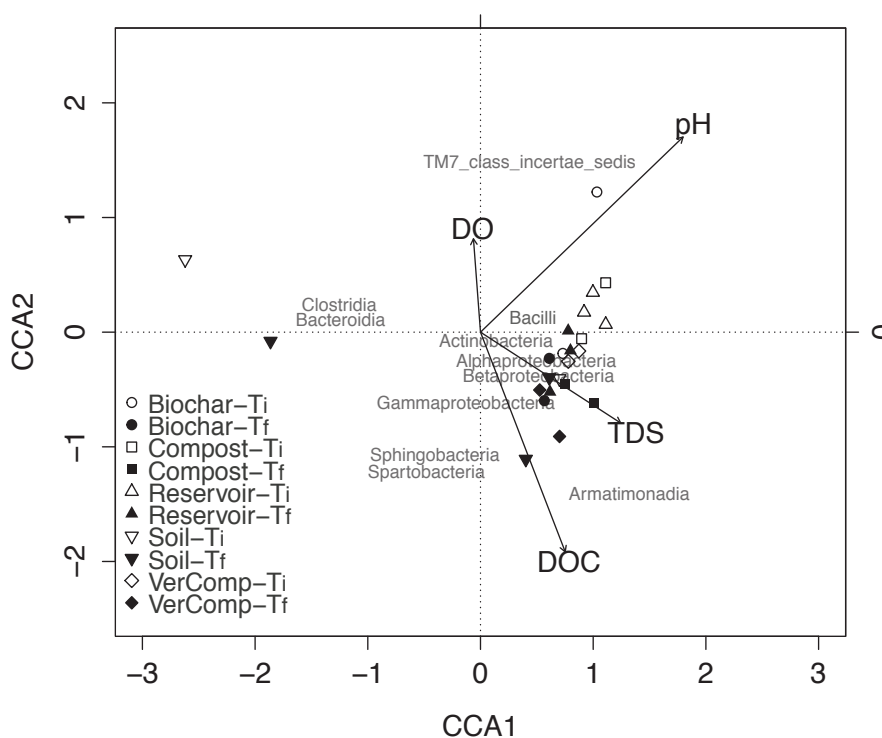


Fig 3.4: Biplot of canonical correspondence analysis of taxa abundance with environmental parameters in all samples at class level. Class of maximum abundance > 1% at least in one sample is included in the analysis.

Taxonomy of core micro-biomes

Of the original 327 327 raw sequences, 17 017 OTUs remained after data processing. Core OTUs were OTUs which abundances constituted > 1 % of the total OTU abundance in at least one sample. The core community represented in all samples encompassed 136 OTUs but 27 % of those could not be classified at the class level.

Due to the large variation among the replicated mesocosms, the differences in abundance of most phyla were not statistically significant among the treatments. A comparison and cluster analysis of microbial composition in the pool samples was performed to assess dominances at the genus level (Fig 3.5a). At T_i , *Pseudomonas spp.* (*Proteobacteria*), *Propionibacterium spp.* (*Actinobacteria*) and *Methylobacterium spp.* (*Proteobacteria*), *Faecalibacterium spp.* (*Firmicutes*) and *Prevotella spp.* (*Bacteroidetes*) dominated the communities in the soil-only runoff incubations with the relative abundance of 10 %, 5.8 %, 6.11 %, 6.8 % and 8.9 % respectively. *Oceanobacillus spp.* (*Firmicutes*) (8 %) were also highly abundant in the biochar runoff incubation whereas *Propionibacterium spp.* were intermediately abundant in the mesocosms that had received compost (2.27 %) or vermi-compost (5.9 %) runoff, or in the reservoir water controls (2.5 %). *Methylobacterium spp.* were also abundant in the

mesocosms with both compost and vermi-compost runoff additions with the relative abundance of 1.84 % and 3.19 %, respectively ($P < 0.05$) (Fig 3.5a).

After 16 days of incubation, clear differences could be observed in bacterial community structure. The relative abundance of *Propionibacterium spp.* increased in all samples with the highest relative abundance in the mesocosms with biochar and compost runoff addition (18.6 % and 17.6 % respectively) and *Pseudomonas spp.* was found with higher relative abundance in reservoir water control (4.89 %) and soil-only runoff incubation (11.2 %) compared to mesocosms with organic fertilizers addition at both T_i and T_f . The relative abundance of *Oceanobacillus spp.* increased sharply in the reservoir water mesocosms by 88.9 % but decreased in those that had received the biochar runoff by 77.4 %. On the other hand, *Methylobacterium spp.* were present in high abundance in the mesocosms that were impacted with runoff water from the compost treatment (8.55 % relative abundance), and they rose in the mesocosms that had received compost or biochar runoff, or in the reservoir controls and decreased in the soil control compared to those at T_i ($P < 0.05$). The genera of *Faecalibacterium spp.* and *Prevotella spp.* also increased and still dominated the communities in the soil only runoff mesocosms at T_f compared to other treatments (Fig 3.5b).

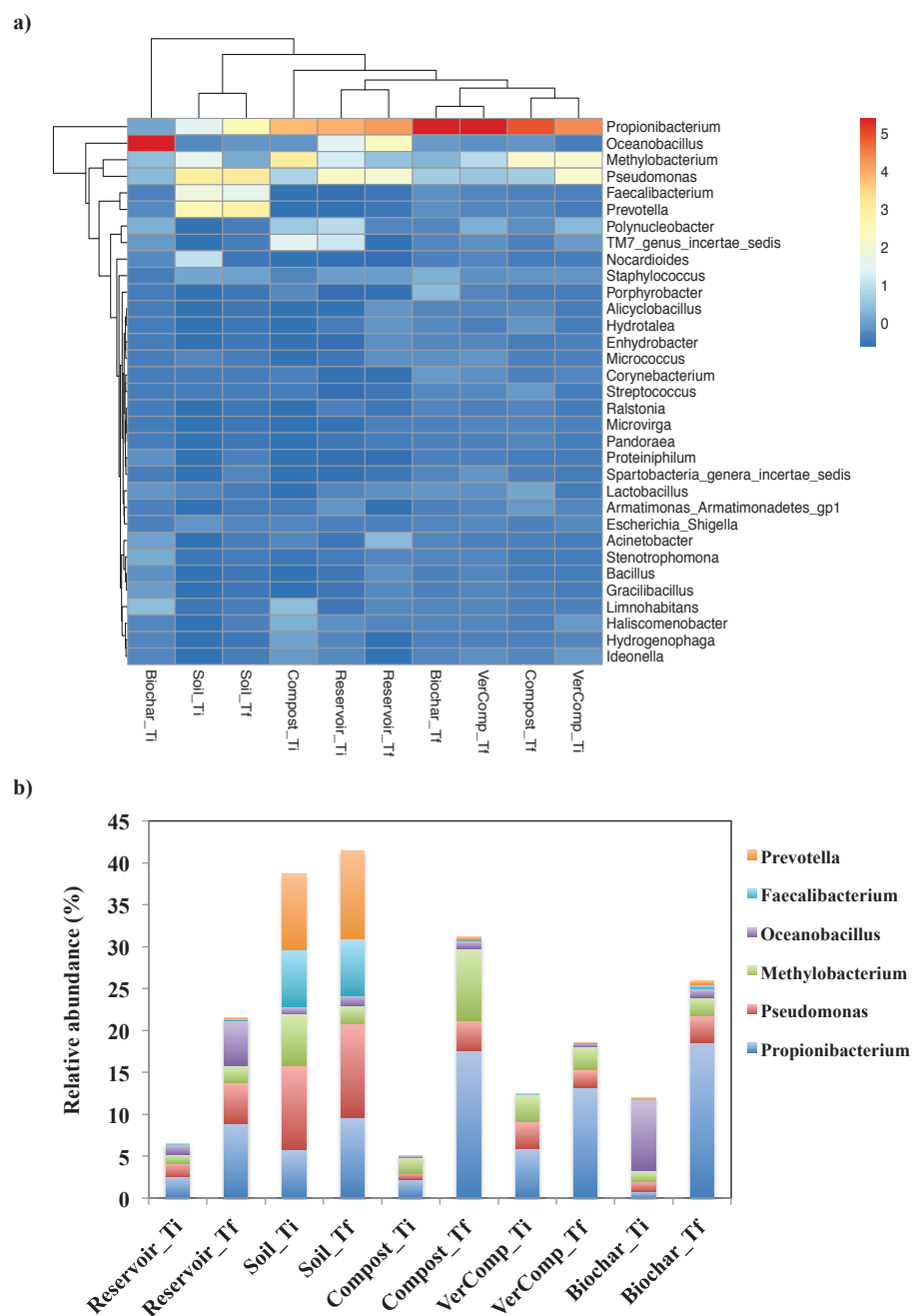


Fig 3.5: a) A heatmap showing the comparison and cluster analysis of microbial composition in the pool samples. Indicator scores are based on the OTU abundance in the microbial assemblages. Only the OTUs with a frequency of > 1% in at least one sample were shown in the figure and b) Comparison of abundance of some genera in all treatments between Ti and Tf.

3.4. Discussion

We conducted a 16-day mesocosm incubation investigation into the impact of organic fertilizer runoff derived DOC on the structure of an aquatic microbial community. Although less ‘naturalistic’ than *in situ* environments, we show that the addition of DOC from organic amendments can alter the structure of microbial communities in such systems, reducing evenness and richness as compared to a reservoir water control. Performing realistic rain application rates on soil parcels under usual fertilizer application rates of compost, vermi-compost and biochar, we found significantly higher DOC concentrations in runoff relative to that of the soil control. Previous studies have also shown that the application of organic carbon from different sources into soil increased the concentration of DOC in surface runoff compared to DOC runoff from bare soil. For example, Gandolfi et al. (2010) observed increases of DOC concentration of 6 to 17 times in surface runoff when corn residues were applied into soil. Janeau et al. (2014) also observed lower DOC export rates in bare soils from the Dong Cao catchment area relative to those with mulch or plant cover.

The addition of runoff to reservoir water resulted in some variability at T_i in both DOC concentrations and in bacterial diversity as a consequence of the different additions. However, in comparison to these allochthonous inputs of DOC at the start of the experiment, the increases in DOC concentration after day 5 were probably from autochthonous sources e.g. primary production as there was a large increase in *Chla* (up to $72.8 \mu\text{g L}^{-1}$) over the first few days. This then declined (Fig 3.2b), suggesting the crash of an algal bloom. The excretion of DOC by phytoplankton during photosynthesis is one of the main sources of DOC in aquatic systems (Mague et al., 1980; Biersmith and Benner, 1998). Indeed, given the high concentrations of *Chla*, it is perhaps not surprising that in some incubations DOC concentration almost doubled. DOC is released during active photosynthesis (exponential growth) but also when phytoplankton biomass becomes stable (stationary phase) or when nutrient limitation and cell death occurs (Biersmith and Benner, 1998; Rochelle-Newall et al., 2008; Rochelle-Newall et al., 2014). This may explain why no direct correlation was observed between *Chla* and DOC concentration. Similar decoupling of DOC dynamics and *Chla* concentration has been observed in other systems: Sommaruga (1999) found no significant correlation between DOC concentration and *Chla* in an Austrian lake. Kim et al. (2000) working in Lake Soyang also reported that the high DOC concentrations observed were due to the presence of cyanobacterial blooms.

Autochthonous vs allochthonous DOC as a structural factor for bacterial communities

Over the course of our experiment, the richness and the evenness of bacteria in mesocosms receiving runoff water from soil amended with organic fertilizers decreased in parallel to an increase in DOC concentration. The only exception was in the mesocosms that had received compost runoff addition where evenness increased. In contrast, the richness of the bacterial communities in the reservoir water and soil only treatments remained unchanged and evenness of the communities increased between T_i and T_f

(Fig 3.3). DOC serves as an important substrate and energy source for heterotrophic bacteria (del Giorgio and Davis, 2003; Wei Tian, 2015a) and the metabolic activity of the community is controlled by DOC quantity and quality and, hence, carbon source (Pommier et al., 2014). In our experiment, the DOC additions in the reservoir water were principally from phytoplankton production (an autochthonous source), whereas in all the other treatments, DOC inputs were initially from soils (allochthonous) and after 6 days from primary production (autochthonous). Thus, the autochthonous vs. allochthonous ratio of carbon source may be an important structural factor influencing bacterial community composition during the incubation. Such results are coherent with those from Attermeyer et al. (2014) who did not observe shifts in bacterial community composition with increasing additions of autochthonous DOC, but did observe a shift when both allochthonous and autochthonous sources were present. Indeed, it has been suggested that bacterial taxa partition along a C source gradient with different ratios of allochthonous vs. autochthonous C (Kritzberg et al., 2006a; Jones, 2009). Evenness in the reservoir water and soil only treatments also increased, which may represent the stimulation of bacterial species specialized in phytoplankton derived organic matter, which are generally considered as highly bioavailable (Rochelle-Newall et al., 2008; Rochelle-Newall et al., 2011). Interestingly, richness also decreased in the mesocosms with biochar, compost and vermi-compost runoff additions, suggesting a specialization of species when both carbon sources were present. Regarding the biochar addition, some bacteria species have been hypothesized to preferentially degrade more recalcitrant soil OM constituents (Anderson et al., 2011) and this may well explain the decreased richness observed in this addition.

Changes in community structures and key taxa correlated with DOC decomposition

Community structures were affected by the incubation although this seemed less marked in the soil-only control incubations (Fig 3.4). DOC and pH changes related to these incubations appear to be the main factors that affected community structure. For example, the relative number of sequences assigned as *Actinobacteria* increased with increasing DOC and pH during the course of the experiment. Similarly, the relative number of sequences representing *Alphaproteobacteria*, *Gammaproteobacteria*, and *Betaproteobacteria* groups were positively correlated with DOC. Previous studies also indicated that pH is an important predictor of bacterial composition and distribution at the continental scale (Lauber, 2009). The relative abundance of *Proteobacteria* groups has been shown to be positively correlated to carbon availability (Fierer et al., 2007a) and to soil pH and organic C concentrations (Wei Tian, 2015a; Wei Tian, 2015b). Similarly, Wei Tian (2015a) also showed that *Alphaproteobacteria*, *Gammaproteobacteria*, and *Betaproteobacteria* groups significantly increased in compost amended soil. The *Actinobacteria* phylum was always predominant across many different finished composts (Franke-Whittle, 2009). *Actinobacteria* are considered important during composting process because of the ability of this group to decompose cellulose and chitin. Moreover, they are considered to play a crucial role in the bio-stabilization of refractory organics (Vivas et al., 2009; Federici E, 2011), the degradation

of recalcitrant compounds and, the suppression of pathogenic microorganisms through the secretion of various antibiotic compounds (Franke-Whittle, 2009).

Two key genera were found to respond to the different treatments during the incubation. At T₆ there was a significant increase in *Propionibacterium spp.* in all treatments, especially in the biochar runoff incubation where the highest number of sequences was found. *Propionibacterium spp.* are a group of gram-positive bacteria that are generally considered as anaerobic to aerotolerant but that prefer anaerobic conditions for growth. *Propionibacterium spp.* utilizes large and complex molecules and convert them into smaller molecules. The production of propionic and acetic acids during lactic acid metabolism is one such example (Wang and Yang, 2013). Interestingly, Gomez et al. (2014) indicated that the addition of biochar in soil shifted the microbial community towards gram-negative bacteria relative to other types of microorganisms, further supporting the suggestion that biochar, although bringing a relatively low addition in terms of DOC, did exert a strong role on bacterial community composition when added to reservoir water. *Methylobacterium spp.* was also dominant in the high DOC concentration compost treatment. These bacteria gram-negative *Alphaproteobacteria* are known to be abundant in compost (Danon, 2008) as well as in soils, lakes and sediments (Rekadwad, 2014). The genus *Methylobacterium* is known to utilize methane and other more complex organic compounds (Yim et al., 2014). Propionic acid, which is produced by *Propionibacterium spp.*, can be utilized as a sole carbon source by *Methylobacterium spp.* (Gallego et al., 2006) indicating a potential metabolic relationship between these two genus. *Propionibacterium spp.* and *Methylobacterium spp.* may thus represent sentinel species for ecosystem changes, particularly in systems with high inputs of allochthonous organic matter (Salvador Pedro et al., 2001).

3.5. Conclusion

Understanding the off-site impacts of agricultural activities is critical if we are to preserve the ecological integrity of managed ecosystems. Our research indicates significant effects of organic fertilizers on the downstream aquatic microbial community in a tropical system. Here we show that realistic amounts of runoff from amended and non-amended soils can provide a large source of allochthonous DOC. In addition, that this allochthonous DOC has a strong impact on microbial community structure and composition in an experimental aquatic system. We found a reduction in both the richness and evenness of the communities with added allochthonous organic matter which may point towards a specialization of the communities, similar to what was previously observed in a short term (72h) incubations (Pommier et al., 2014). Moreover, the data suggest that DOC and pH are the main factors controlling community structure in mesocosm experiments. Ultimately, we identified two sentinel genera, *i.e.* *Propionibacterium spp.* and *Methylobacterium spp.*, which could be important to track using e.g. targeted quantitative PCR, to monitor ecological changes in sites under high OM inputs. These results further underline that even though organic composts can be considered as being less damaging for

the environment as compared to other chemical additions, the full consequences of their application need to be taken into account, especially in agricultural practices of tropical countries.

In chapter 3, I showed the response of a bacterial community from a reservoir to additions of runoff from soils amended with different organic carbons in mesocosm experiment incubated of 16 days incubation. To more realistically assess the effects of terrestrial runoff into aquatic DOC and bacterial diversity and functioning, taking into account hydrological variations, another investigation was conducted *in situ* in the Houay Pano catchment, Laos (Chapter 4 and 5). Compared to the change in fertilization regime investigated in Vietnam, this catchment experienced rapid LU change from annual crops (upland rice) to teak plantation, which is of particular concern in tropical regions.

In the next chapter, I shall explore whether LU change from upland rice to teak plantation and agricultural practices of burning understorey under teak plantation caused more soil erosion and degradation by assessing the loss of soil organic carbon and soil particle attached (PA) and free-living (FL) fractions in the runoff water following a simulated rain.

CHAPTER 4
LAND USE STRONGLY INFLUENCES SOIL ORGANIC CARBON
AND BACTERIAL COMMUNITY LOSS IN RUNOFF IN TROPICAL
UPLANDS

This chapter is in preparation for submission to journal “Land degradation and development “

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Abstract

Land degradation and soil erosion are serious issues especially in tropical ecosystems where land use (LU) changes are occurring rapidly. Soil erosion under heavy rainfall can cause loss of soil organic carbon (SOC) and the associated bacterial community through runoff, which in turn affects soil functioning and productivity. The aim of this study was to assess land degradation (loss of SOC and soil bacterial diversity and functions in surface water runoff) from three different LU: Teak with/without understorey (TW/TWO) and upland rice (UR) in a tropical, upland catchment during a simulated rain event. The runoff coefficient and total suspended sediment (TSS) were highest in TWO but total organic carbon (TOC) in TSS and dissolved organic carbon (DOC) concentration was lower in runoff water from this LU compared to TW and UR. This suggests that burning understorey under teak plantation causes loss of organic carbon through removal of vegetation debris. Moreover, the highest proportion of bacterial taxa common to soil and runoff water was identified in runoff water from TWO as compared to TW and UR for both particle attached and free-living fractions. More importantly, bacterial community structure loss in surface runoff was driven by change in DOC and TSS concentration, suggesting the eroded soil particles simultaneously carry organic carbon and attached bacterial taxa in the surface runoff. Consequently, loss of soil functional group relating to organic carbon degradation and nitrogen cycles was higher under TWO than the other LUs. Our results underline that teak plantation with unsustainable practices of burning understorey generate degraded soil function and productivity and accelerated land degradation through soil erosion and surface runoff.

Keywords: Unsustainable land use, land degradation, soil erosion, rain simulation, organic carbon, bacterial function loss.

4.1. Introduction

Agricultural land degradation, especially soil erosion, is a serious environmental problem, especially in developing tropical countries (Valentin et al., 2008). Compared to temperate regions, tropical regions are facing more soil erosion and land degradation due to high intensity rainfall and agricultural intensified LU, especially in marginal steep lands (Labrière et al., 2015). For example, the erosion rate in hilly croplands under conventional agriculture in the tropics was higher (50-100 tons ha⁻¹ yr⁻¹) than in the temperate zone (10-20 tons ha⁻¹ yr⁻¹) (FAO, 2015).

Land use change is one of the most important factors causing land degradation through accelerating surface runoff and soil erosion (García-Ruiz, 2010; Vanwalleghem et al., 2017; Guzha et al., 2018). Hill and Mervyn (1998) in the review of links between LU, runoff and soil erosion in Southern China found that the average erosion rate in cultivated slopes of 62.4 t ha⁻¹ year⁻¹ was much more higher than in forests and woodlands (0.05 t ha⁻¹ year⁻¹). Guzha et al. (2018) indicated that forest cover loss increased annual discharges and surface runoff by 16 % and 45 % respectively. Moreover, vegetation cover on the surface soil is very important in controlling erosion. Clearing vegetation leads to soil compaction, surface crusting and reduced water infiltration and exposes soils to erosion (Baker and Miller, 2013).

The expansion of agriculture has accelerated soil erosion significantly, mobilizing around 783 ± 243 Pg of soil organic carbon (SOC) globally over the past 8000 years (Wang et al., 2017d). The export of sediment – associated OC is higher in cultivated lands (266.4 kg ha⁻¹ y⁻¹) than other lands such as grazing lands (74.5 kg ha⁻¹ y⁻¹) and tree plantations (6.2 kg ha⁻¹ y⁻¹) (Gebresamuel et al., 2010). Walmsley et al. (2011) found that higher leaching loss of organic carbon from non-inversion tillage plus crop reduced potential carbon gain of many croplands by up to 20 g m⁻² y⁻¹, thus affecting carbon sequestration in cropland soils.

The soil bacteria community is responsible for most soil biological transformations and their activity drives the cycling of carbon, nitrogen and other nutrients (Schulz et al., 2013). Any changes in diversity and activity of soil microorganisms can affect soil functions relating to organic matter and nutrient cycling and soil productivity (Araujo et al., 2010). Therefore the loss of bacterial community through runoff water can negatively affect soil functions and productivity. However, most studies to date have tended to focus on the export of fecal bacteria from soil into runoff and the risk of surface water contamination in both temperate (Jamieson et al., 2002; Muirhead et al., 2006) and tropical systems (Rochelle-Newall et al., 2015). To our knowledge, little work has focused on the understanding of land degradation through the loss of diversity, structure and function of the whole bacterial communities from the soil surface in surface runoff during rain events in tropical systems subject to rapid LU change.

The objective of this study was to assess land degradation relating to loss of organic carbon and soil bacterial diversity and function into surface runoff under effect of different LU including Teak with or without understory (TW or TWO, respectively) and upland rice (UR) in a tropical, upland catchment in Northern Laos. To understand the movement of organic carbon and bacterial community through runoff water, a rainfall simulation was performed under different LU because this is a powerful and widely used tool for investigating runoff, soil erosion processes and soil water movement under different conditions (Zhao et al., 2014; Le et al., 2016; Zemke, 2016).

The studied area has in the past decade trajected from upland crops (e.g. upland rice) to teak plantations due to economic development and government LU policy. The transition from rice-based shifting cultivation to teak plantation caused an increase of the overland flow contribution to the stream from 16 to 31% and sediment yield from 98 to 609 Mg km⁻² (Riboldi et al., 2017). Teak trees have large leaves which lead to the formation of high kinetic energy raindrops under heavy rain, the drops increase the splash effect, blocking soil surface porosity and destroying the soil surface (Assouline and Mualem, 1997; Lacombe et al., 2017). This leads to higher surface crusting and lower infiltration rates under teak compared to rice cultivation (Riboldi et al., 2017). We hypothesized that teak plantations lead to a higher loss of soil organic carbon and bacterial richness and diversity in runoff water than under upland rice. In addition, the cultivation practice of burning the understory in the teak plantations (TWO), used to limit the competition between understory species and teak plantation (Patin et al., 2012) was also examined. The removal of ground vegetation results in the soil surface being exposed to higher kinetic energy raindrops leading to the formation of an impermeable surface crust and increased overland flow (Riboldi et al., 2017). Therefore, we hypothesized that between the two teak plantation practices, TWO will lead to increased export of DOC and bacterial community into surface runoff as compared to that with an intact understory (TW).

4.2. Material and methods

Study site and LU

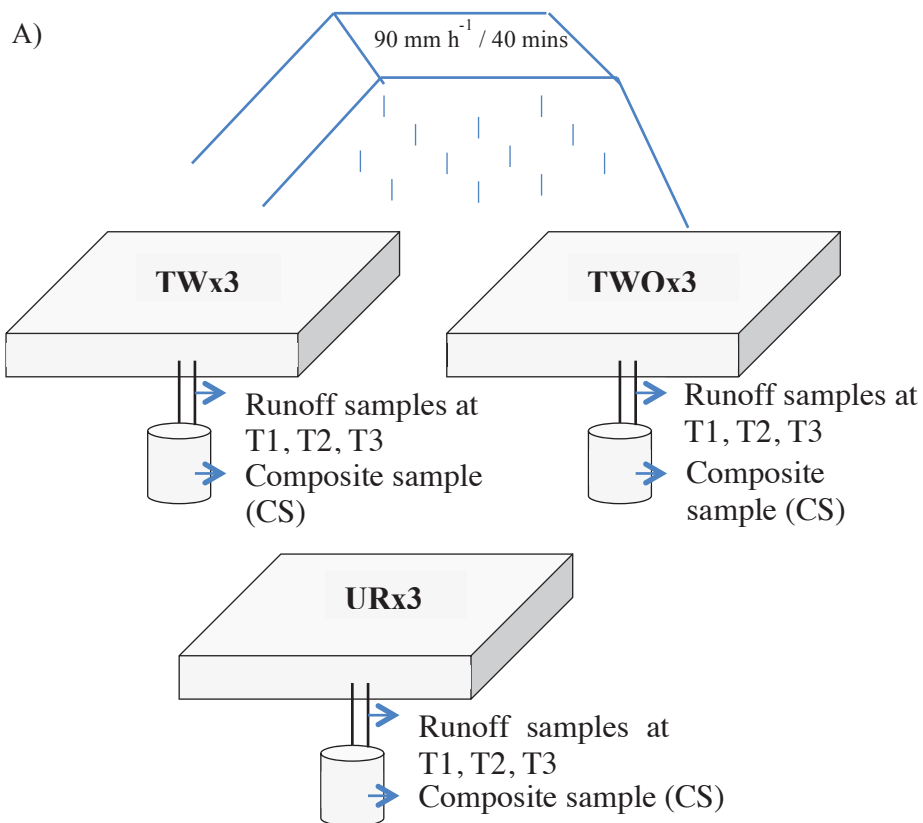
The rain simulation experiment was carried out in the Houay Pano catchment area (19°51'N–102°10'E), located 10 km south of Luang Prabang in northern Laos in the Mekong river basin. The area is characterized by a tropical monsoon climate with a wet season from April to September and a dry season from October to March. The average annual precipitation is 1,585 mm year⁻¹, more than 80% of which occurs during the wet season (Riboldi et al., 2008). Mean annual temperature is 25.3°C (Riboldi et al., 2011).

This catchment is representative of the rapidly changing LU from shifting cultivation with crop fields to planted forests, notably teak forests, in Southern Asia. Moreover this catchment has been monitored since 2001 and during this time the area occupied by teak plantations has increased from 20% in 2008 to nearly 40% area in 2012 (Huon et al., 2013). In 2015, teak plantation covered 23.87 ha

(35.7%), upland rice covered 1.18 ha (1.76%), forests covered 5.53 ha (8.27%), and fallow covered 31.16 ha (46.6%) (Le et al., 2018). The remaining areas are covered by vegetable gardens, banana and other fruit trees, maize, Job's tears, and sweet potato.

Rain simulation, sample collection and major physical and chemical characteristics

The rainfall simulation was applied to 1 m² plots following the method described in Janeau et al. (2014) under different LU including: teak with understory (TW), teak without understory (TWO) and upland rice (UR) with three replicate plots (A-B-C) for each LU (Fig 4.1).



B)



Fig 4.1: A) Rain simulation experiment under different land use. B) Example of rain simulation used under teak without understory (TWO). Runoff waters from the exit tube of the 1m² plot were determined every 30 sec using 100mL plastic bottle in order to calculate runoff volume during the rain simulation. Concomitantly with the runoff volume, samples were collected three times at T1, T2 and T3 during the rain simulation and a composite sample (CS) was collected from the bucket at the end of the rain simulation for measurement of dissolved organic carbon (DOC), colored dissolved organic matter (CDOM), Total suspended sediment (TSS) and total organic carbon (TOC) in TSS and bacterial functioning and diversity

Each plot was formed from the enclosure of a 1m² area within a steel plot frame. The square plot frames have metal sides of 20 cm high and are 1 m long on each side. Each plot was embedded approximately 10 cm into the soil surface and each frame was placed at between 4 and 6 m distance from the adjacent plots to ensure that there was no secondary influence from the other rain simulations. A rain intensity of 90 mm h⁻¹ and a duration 40 min (~ 585 J m⁻²) was used for each simulation. This intensity corresponds to the median intensity of monsoon rain event observed over a 10 year period in this region (Janeau et al., 2014). A sample of rainwater (RW) was collected to provide a background measurement. Runoff water from the soil surface was drained from the bottom side of the plot via a drainage channel and was collected, via a hole in the bottom of the frame, in a large, clean bucket during each rain event. Samples (~1.5L) were collected three times (T1, T2, T3) during the rain simulations for the measurements of DOC, colored dissolved organic matter (CDOM), Total suspended sediment (TSS) and total organic carbon (TOC) in TSS and bacterial functioning and diversity. A composite sample (CS) was also collected from the bucket at the end of the rain simulation, hence comprising all waters not collected at T1-T3.

The runoff coefficient was calculated for each LU using the following formula:

$$\text{Runoff coefficient (\%)} = \frac{\text{Total runoff volume}}{\text{Total rainfall volume on the 1m}^2 \text{ plot}} \times 100$$

Three soil cores (0-10 cm) from each LU: teak with understorey (TW), teak without understorey (TWO) and upland rice (UR) was collected outside each plot. All soils were characterized as alfisol (Chaplot, Rumpel, & Valentin, 2005). All soil cores were transported to the laboratory where they were homogenized by 2-mm sieving and roots were removed.

Measurement of DOC, CDOM, TSS and TOC - TSS

A subsample of 200 mL of each sample was filtered through Whatman GF/F glass fiber filters to remove particles. For the determination of DOC concentration, duplicate 30 ml of this filtrate was kept in pre-combusted (450°C, overnight) glass tubes, preserved with 36 µL 85% phosphoric acid (H₃PO₄) and sealed with a Teflon lined cap. Samples were stored at ambient temperature and in the dark until measurement. DOC concentration was measured on a Shimadzu Total Organic Carbon (TOC) V_{CPH} analyzer following the method described in Rochelle-Newall et al. (2011). DOC concentration is expressed as mg of organic C per liter of water.

For CDOM, 100-ml filtered samples were stored in pre-cleaned 125-ml amber glass bottles sealed with Teflon lined caps. After collection, the samples were stored frozen (-20°C) until measurement. Before the optical measurements, the samples were thawed slowly to room temperature and re-filtered at 0.2 µm (Sartorius Minisart NML Syringe filters) to remove particles. CDOM absorption

was measured with a spectrophotometer (Analytica.Jena Specord 205 UV-VIS) from 200-750 nm using a 10 cm quartz cell and Milli-Q water as the blank.

SUVA₂₅₄ (specific UV absorbance) provides a measure of aromaticity of DOM (Weishaar et al., 2003) and was computed by dividing the UV absorbance at 254 nm (m^{-1}) by the concentration of DOC ($mg\ C\ L^{-1}$) (Hood et al., 2006). The spectral slope ratio, S_R was also calculated as the ratio of the slope of the shorter UV wavelength region (275–295 nm) to that of the longer UV wavelength region (350–400 nm) (Helms et al., 2008) and was obtained using linear regression on the log-transformed spectral ranges (Yamashita et al., 2010). Humification (HIX) was calculated from excitation 255 nm as the ratio of the peak area under each curve at emission 434-480 nm and 300-346 nm. HIX indicates the humic content of DOM and ranges from 0 to 1 (Ohno, 2002).

Excitation – Emissions – Matrice (EEM) measurements were made on a Gilden Fluorosens fluorometer using a 1 cm quartz cuvette with 5 nm bandwidths for excitation and emission at an integration time of 100 ms. Excitation scans were made over a range of 200 to 450 nm at 5 nm increments and emission from 220 to 600 nm at 2.5 nm increment. EEMs were corrected for inner filter effects and the manufacturers' machine correction was applied. EEM fluorescence of Milli-Q water blank was subtracted from that of sample EEM, and EEM converted to RU units.

PARAFAC analysis was carried out in MATLAB (version R2016a 9.0.0) with the DOMFluor toolbox for MATLAB (Murphy et al., 2013) in order to decompose the fluorescence signal into a series of tri-linear structures. Processing data was done to minimize the impact of scatter lines that caused some mathematical difficulties, including removal of Rayleigh and Raman scatter. EEM wavelength ranges were reduced to excitation 290-450 nm and emission 300-600 nm. PARAFAC model generated 3 components using EEMs from our samples (n=37). The three-component model (C1-C3) was validated using split-half and random initialization methods.

Total suspended sediment (TSS) samples were ground with an agate mortar, weighed and packed into tin containers (5x9 mm). Total organic carbon (TOC) were measured on the TSS samples using the Elemantar VarioPyro cube analyzer on line with a Micromass Isoprime Isotope Ratio Mass Spectrometer (IRMS) following the method described in Huon et al. (2017).

Carbon metabolic capacities of bacterial communities

Biolog[®] Ecoplate was used to determine the catabolic capacity of bacterial communities. These 96-well micro-plates include triplicates of 31 different carbon-based substrates and one water control. Each well also contains a tetrazolium violet dye that turns purple upon substrate oxidation (Stefanowicz, 2006). One ml water sample was diluted 10 times with 0.2 μm filtered stream water to avoid osmotic shock and allow detection of color changes. One hundred and fifty (150) μl of this diluted sample was added to each well, and the microplates were then incubated in the dark at 25 – 27°C without shaking. Following a short shaking (2 secs at 100 rpm) well color development was measured at 590 nm using a

portable spectrophotometer (Bio-Rad laboratories, iMark Microplate Reader) after 12 hours incubation and then every 24h for 96h. The water blank was subtracted from the carbon substrates, which were then grouped into 6 substrate families (amine, polymers, phenolic acids, carboxylic acids, carbohydrates, amino acids) (Pommier et al., 2014). Metabolic potential of each community was normalized to each substrate family relative to the highest performance in the substrate family.

Assessment of bacterial diversity

DNA extraction and 16S rRNA gene sequencing

Fifty milliliters of each sample was first filtered through a 3 μm pore size filter (Polycarbonate Whatman) and a 0.2 μm (Polycarbonate Supor) filters to separate Particle-attached (PA) fraction ($>3.0 \mu\text{m}$) from Free-living (FL) fraction ($<3.0 \mu\text{m}$ and $>0.2 \mu\text{m}$) (Crump et al., 1999). All filters were stored at -20°C until DNA extraction that followed a modified protocol adapted from Fuhrman et al (1988). Briefly, the filters were cut in two halves using ethanol cleaned scissors and placed in separate Eppendorf[®] tubes. One tube was placed on ice and 525 μl of lysis buffer was added to start breaking Gram negative bacterial cells. The other tube was stored at -20°C as a back-up filter. Three cycles of freeze-thaw switches (65°C for 2 min – ice for 5 min) were then performed to facilitate cell membrane breakage. Then 0.5 g of glass beads ($\text{Ø} = 0.5 \text{ mm}$) was added into the tubes. The tubes were shaken for 45 s at 6 ms^{-1} using a Fastprep[®] (Millipore, Fastprep[®]-24, USA) and were incubated at 4°C for 5 mins; these steps were performed twice. 11 μl of Lysozyme (1 mg mL^{-1} , final concentration) was added to the tube and left for 30 min at 37°C to break down bacterial cell walls. Sodium dodecyl sulfate (SDS 10%) and proteinase K (final concentration, $100 \mu\text{g mL}^{-1}$) were added to the tubes and were incubated at 55°C for 2 hours under constant shaking conditions (180 rpm) to remove the lipid membrane. A sodium chloride (NaCl 5M) and a cetyltrimethylammonium bromide (CTAB) solution (final concentration, 1% in a 0.7 M NaCl solution) was added to the tubes, mixed and incubated at 65°C for 10 min to separate DNA from protein. Nucleic acids were then extracted twice from digestion products with phenol-chloroform-isoamyl alcohol (25:24:1); the aqueous phase containing nucleic acids was kept and purified by adding phenol-chloroform-isoamyl alcohol (25:24:1). After isopropanol addition, the nucleic acids were precipitated at -20°C for 12 h. After centrifugation, the DNA pellet was rinsed with 99% pure ethanol to remove the salt previously added. The samples were spun 20 min at maximum speed (15,000 rpm) at 4°C and the supernatants were removed. The DNA pellets were dried in a Speed Vac[®] (Labconco, USA) for 10 min and re-suspended in 50 μl of molecular cleaned distilled water. Nucleic acid extracts were stored at -20°C and sent overnight to Molecular Research Laboratories (Texas, USA) for further PCR amplification, product cleaning, library construction and high throughput sequencing.

Soil DNA was extracted from 0.5 g of frozen sieved soil using the Power Soil TM DNA Isolation Kit (MO BIO laboratories, Carlsbad, CA, USA) following the manufacturer's instructions.

The PCR primers 515F (5'-AGRGTTTGATCMTGGCTCAG-3') and (5'-806R GTNTTACNGCGGCKGCTG-3') (producing a 291bp amplicons) (Capone et al., 2011) with sample-specific barcodes on the forward primer were used to amplify the V4 variable region of the 16S rRNA gene. Thirty (30) cycles of PCR were performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 mins, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 mins was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification, the relative intensity of bands and to check the expected amplicon size (291bp). The samples included in this study were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled PCR products were purified using calibrated Agencourt AMPure XP magnetic beads according to the manufacturer's guidelines. Briefly, the pooled PCR products were incubated with magnetic beads (1.8:1 ratio of beads to sample) that pull down the PCR product to the bottom of a tube for subsequent washing steps. The clean PCR product is then release from the magnetic beads for elution. The pooled and purified PCR product was then used to prepare a DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing was performed at MR DNA (www.mrdnlab.com, Shallowater, TX, USA) on an Illumina MiSeq machine following the manufacturer's guidelines.

Sequences processing and data analysis

The MOTHUR software (v. 1.33) (Schloss et al., 2009) was used to process 16S rRNA gene sequence reads following the standard operation protocol. Short reads (<250 bp) and reads with ambiguous primer or barcode sequences were discarded. Corresponding reads were paired in single sequences with an average length of 486 bp. Sequencing errors were reduced by aligning remaining reads to the SILVA database (Pruesse et al., 2007), screening the alignment to the overlapping region, and pre-clustering sequences distant with < 2 bp. Chimeric sequences were identified using the integrated version of UChime (Edgar et al., 2011) and removed accordingly. To avoid misinterpretation, sequences that were classified as "Chloroplast", "Mitochondria", or "unknown" lineages were removed before clustering into Operational Taxonomic Units (OTUs). All samples were subsample into 10,000 sequences before clustering in OTU with a pairwise distance < 0.03 substitutions per nucleotide with average neighbor method and considered for further analyses. Taxonomic assignments were performed on the alignment of consensus sequences with the RDP database (Cole et al., 2005).

The sequences that were considered to be contamination from rainwater (i.e. also found in the RW sample) were removed from corresponding runoff samples before further analysis.

Chao 1 (richness), Invsimpson (evenness) and Shannon index was calculated for runoff samples at each time point for each LU during the rain simulation using MOTHUR software (v.1.33). We performed alpha diversity calculation based on pooled data from replicate samples to increase the

number of sequences for each sample and to reduce the bias in our estimate of diversity. The number of sequences per sample was sub-sampled at 8,989 sequences for the alpha diversity calculation.

A nonmetric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity was also calculated to estimate the dissimilarity in structure between all samples using the *metaMDS* and *ordisurf* functions from the *vegan* package in R studio (Oksanen et al., 2017). Venn diagrams were built to show shared OTUs between different LU in R studio with the *venneuler* package.

The microbial putative functions were displayed by all OTUs from soil and shared OTUs between soil and surface runoff samples from different LU using FAPROTAX (Louca et al., 2016) to identify the functional groups lost from soil to surface runoff under the rain simulation.

Statistics analyses

ANOVA was used to test significance of the differences between different LU after checking that the assumptions of the ANOVA were met. When necessary, the data were log-transformed to assure normality. If the data did not follow the normal distribution even after transformation, non-parametric Wilcoxon Signed-Rank Test was used to test differences between different LU.

Correlations between DOM concentrations and optical index was estimated using the Pearson correlation coefficient with the *Hmisc* package in R (Harrell, 2018). ANOSIM test was performed with Bray-Curtis dissimilarity using *vegan* package in R (Oksanen et al., 2017) to determine the significance of the difference in bacterial community structure. Also, the *env.fit* function was applied to the Bray-Curtis dissimilarity matrices (*vegan* package) to evaluate the significance of the relationship between particle-attached (PA) and free-living (FL) fractions of the bacterial structure with two abiotic factors (DOC and TSS). All differences were considered significant when the p-value of the tests was inferior to 0.05.

4.3. Results

Characteristic of runoff waters from different LU

During the rain simulation experiment, the average of runoff coefficient was highest in TWO with 28.63 ± 5.8 %, followed by UR (21.69 ± 10.5 %) and TW (11.26 ± 6.2 %) (Table 4.1). Runoff increased over time on each replicate for all different LUs during the course of the rain simulation (Fig 4.2). In parallel, the export of TSS decreased in each replicate of the different LUs, except for TWO. More specifically, TSS in runoff under TWO increased from a range of $0.8 - 1.4$ to $1.01 - 2.05$ g L⁻¹, then this value decreased to $0.83 - 1.53$ g L⁻¹ in the different plots (Fig 4.2). The average values of TSS were higher in TWO (1.23 ± 0.21 g/L) than in TW (0.47 ± 0.16 g L⁻¹) and UR (0.44 ± 0.2 g L⁻¹) (Table 4.1).

Table 4.1: Average of runoff coefficient, total Suspended Sediment (TSS), total organic carbon in TSS (TOC-TSS), dissolved organic carbon (DOC) concentration and its the optical characteristics (CDOM) under different LU: aromaticity (SUVA₂₅₄), the inverse of molecular weight (Sr), humification index (HIX) and three PARAFAC components C1-C3.

	TW	TWO	UR
Runoff coefficient (%)	11.26±6.2	28.03±5.8	21.69±10.5
TSS (g L ⁻¹)	0.37±0.16	1.23±0.21	0.44±0.2
TOC-TSS (mg C g ⁻¹)	65.03±10.8	30.9±2.42	45.5±7.76
DOC (mg L ⁻¹)	13.4±7.5	3.8±0.7	9.57±4.8
SUVA ₂₅₄ (mg ⁻¹ L m ⁻¹)	4.12±0.5	4.23±0.7	5.86
Sr	0.89±0.19	1.22±0.16	0.86
HIX	0.42±0.09	0.4±0.02	0.45
C1	1.48±0.19	1.2±0.15	1.27
C2	2.26±0.46	2.1±1.2	2.38
C3	1.73±0.74	1.45±0.29	0.78

DOC concentration exhibited a decreasing trend with increasing runoff over the duration of the rain simulation for all LU (Fig 4.2). DOC concentration ranged from 3.89 – 6.34, 20.9 - 25.1 mg L⁻¹ and 12.5 – 21.7 mg L⁻¹ at the beginning of the rain simulation and decreased to 2.82 – 3.25 mg L⁻¹, 7.3 – 9.5 mg L⁻¹ and 3.6 – 7.3 mg L⁻¹ at the end of the simulation for TWO, TW and UR, respectively.

In contrast to the runoff coefficient, the average DOC concentration was lowest in TWO 3.8 ± 0.7 mg L⁻¹ followed by UR (9.57 ± 4.8 mg L⁻¹) and then TW (13.4 ± 7.5 mg L⁻¹) (Table 4.1). Similarly, the average of total organic carbon measured in suspended sediment (TOC – TSS) was lowest in TWO (30.9 ± 2.42 mg C g⁻¹) than TW (65.03 ± 10.8 mg C g⁻¹) and UR (45.5 ± 7.76 mg C g⁻¹) (Table 4.1).

Similarly, CDOM optical properties varied considerably between different LU over the course of the rain simulation (Table 4.1). The aromaticity of DOC (SUVA₂₅₄) was higher in runoff under UR 5.86 mg⁻¹ L m⁻¹ than in runoff from TW (4.12 ± 0.5 mg⁻¹ L m⁻¹) and TWO (4.23 ± 0.7 mg⁻¹ L m⁻¹). The humification index (HIX) was rather similar for each LU and varied from 0.42 to 0.45. Sr, the inverse of molecular weight, was highest in runoff from TWO, indicating the molecular weight was lowest under this LU.

A three components model was validated by PARAFAC for the runoff samples. Component 1 has an excitation/emission peak at 395/502 nm and is characterized as UVA humic-like (Ishii and Boyer, 2012). Component 2 (excitation/emission peak 460/345 nm) corresponds to UVC humic-like/high-

molecular-weight humic matter; it is widespread but is found in high relative proportions in wetlands and forested environments (Fellman et al., 2010). Component 3 (excitation/emission peak 305/355nm) can be characterized as a tryptophan component (Yamashita and Tanoue, 2003; Zhang et al., 2010). The humic-like fluorescence components (C1 and C2) were lower in runoff under TWO than that in TW and UR (Table 4.1).

DOC concentration was significantly positively correlated with humification index (HIX) and with components C1 and C2 of the PARAFAC model (Pearson correlation, $R= 0.78$; 0.66 and 0.58 ; $P=0.002$, 0.018 and 0.043 respectively) and negatively with Sr (Pearson correlation, $R=-0.76$, $P=0.004$).

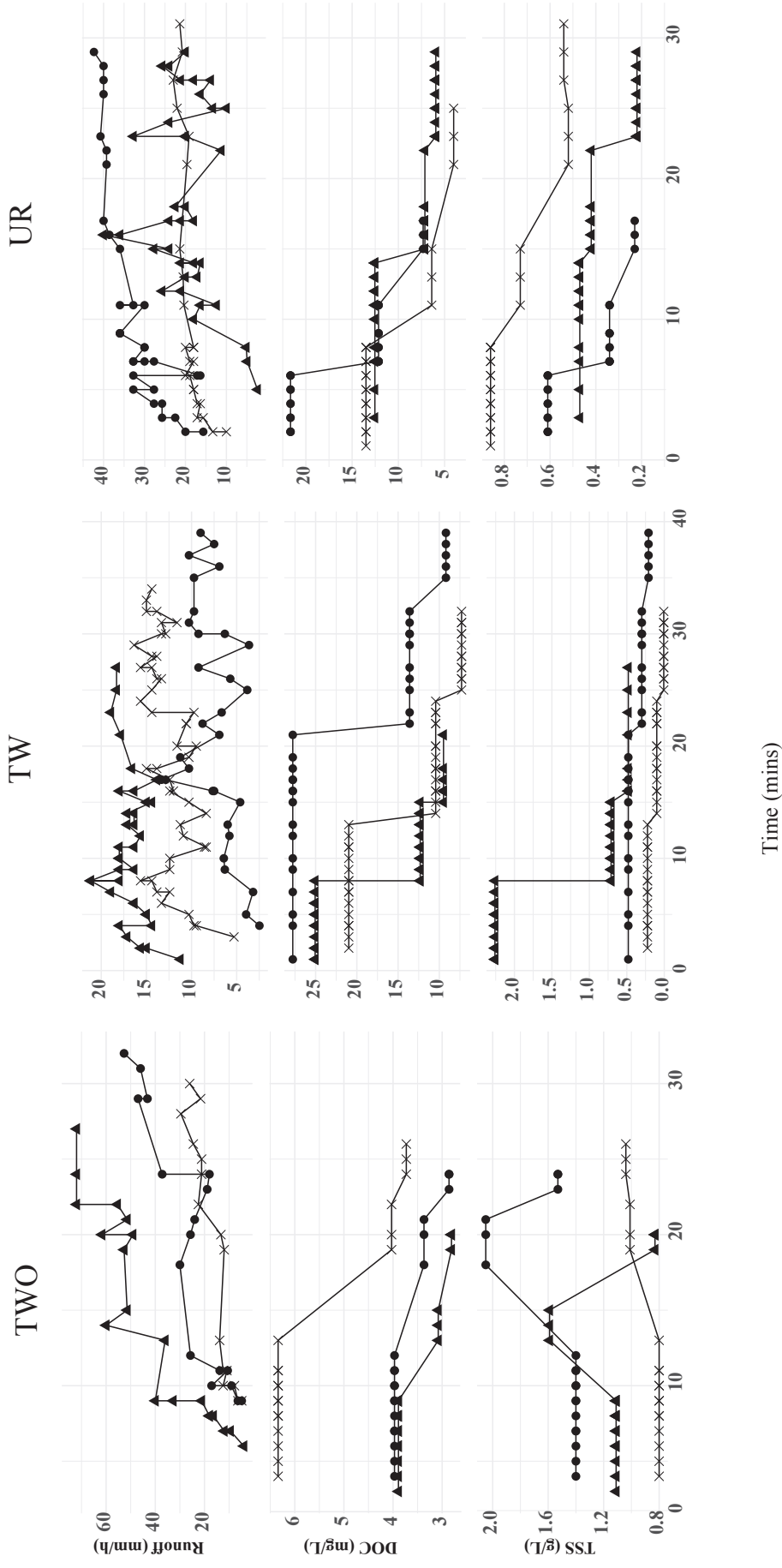


Fig 4.2 Temporal change of runoff, TSS and DOC concentration during the course of the rain simulation for three different LU. The different symbols represent different replicates.

Bacterial diversity, structure, and carbon metabolic potential in runoff waters from different LU

Rarefaction curves indicated that the estimated Chao1 richness of bacterial communities in the runoff waters from TWO were higher than under TW and UR and the diversity of PA were higher than the FL fractions for all runoff samples in different LU (Fig S 4.1).

The evenness (Invsimpson) and the diversity (Shannon index) of PA fraction decreased over time in all three LU. Meanwhile the evenness of FL increased in the runoff from TW and TWO, but decreased in the runoff from UR. In addition, the diversity of bacterial community (Shannon index) was highest under TWO as compared to TW and UR for FL fractions (Table S 4.1).

Bacterial community structure differed significantly (ANOSIM, $p = 0.001-0.048$) between the soil and runoff samples from different LU (Table 4.2). The structure of the PA and FL fractions was significantly separated in runoff samples for each LU (Fig 4.3, Table 4.2). The bacterial community in runoff water samples from TWO, especially for the FL fraction, was closer to soil samples than to TW and UR. In contrast, the PA fraction of the bacterial community from runoff water samples was more similar to soil samples compared to the FL bacterial community in TW and UR (Fig 4.3).

Table 4.2: Result of Analysis of Similarities (ANOSIM) to test for significant differences in bacterial community structure

Sample	Factor	R	P-value
Soil	LU	0.37	0.002**
Runoff	LU	0.55	0.001***
Runoff (TW)	PA&FL	0.84	0.001***
Runoff (TWO)	PA&FL	0.63	0.001***
Runoff (UR)	PA&FL	0.99	0.001***
Soil-runoff (TW.PA)	-	0.97	0.001***
Soil-runoff (TW.FL)	-	0.82	0.001***
Soil – runoff (TWO.PA)	-	0.99	0.001***
Soil – runoff (TWO.FL)	-	0.24	0.048*
Soil – runoff (UR.PA)	-	0.99	0.001***
Soil-runoff (UR.FL)	-	0.9	0.001***

R: explained variance, p-value: level of significance. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

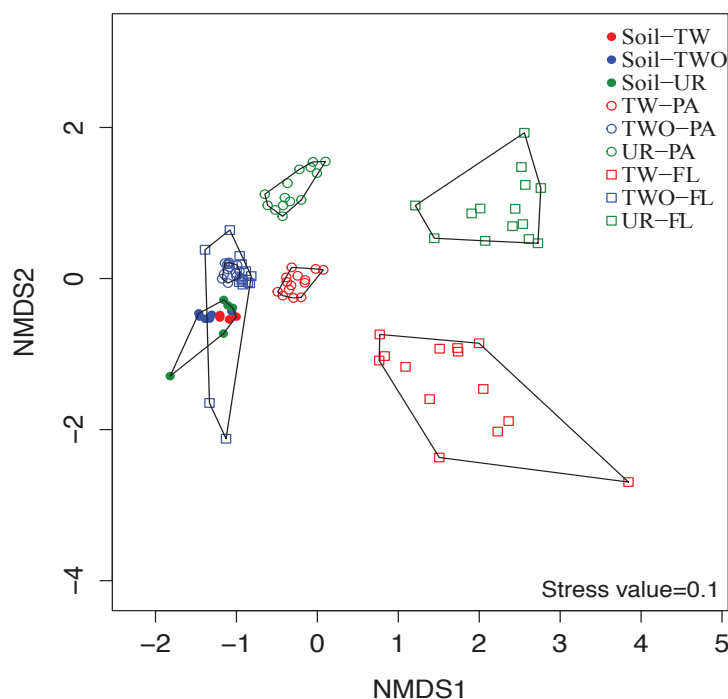


Fig 4.3: NMDS analysis for all samples based on Bray-Curtis dissimilarity. The polygon indicated the different groups according to ANOSIM analysis.

Environmental drivers of bacterial composition were analyzed by fitting selected environmental variables to the ordination matrix of the second NMDS (Fig 4.4). This indicated that DOC and TSS were the two significant environmental factors explaining the structure of the PA and FL fractions of the bacterial community (Fig 4.4, Table S4.2). However, DOC concentration was more related to PA bacterial structure than FL bacterial structure (Table S4.2).

Carbon substrate utilization in the runoff water of the composite samples of the different LU was measured after 96h (Fig 4.5). The results revealed that the metabolic potential of the community, in term of carbon degradation capacity was significantly different between teak plantation (TWO and TW) and UR ($P=0.015$). The carbon utilization of some substrate families including amine, amino acid, carboxylic acids and polymer was lower in the runoff water from UR than in the runoff water from TWO and TW (Fig 4.5).

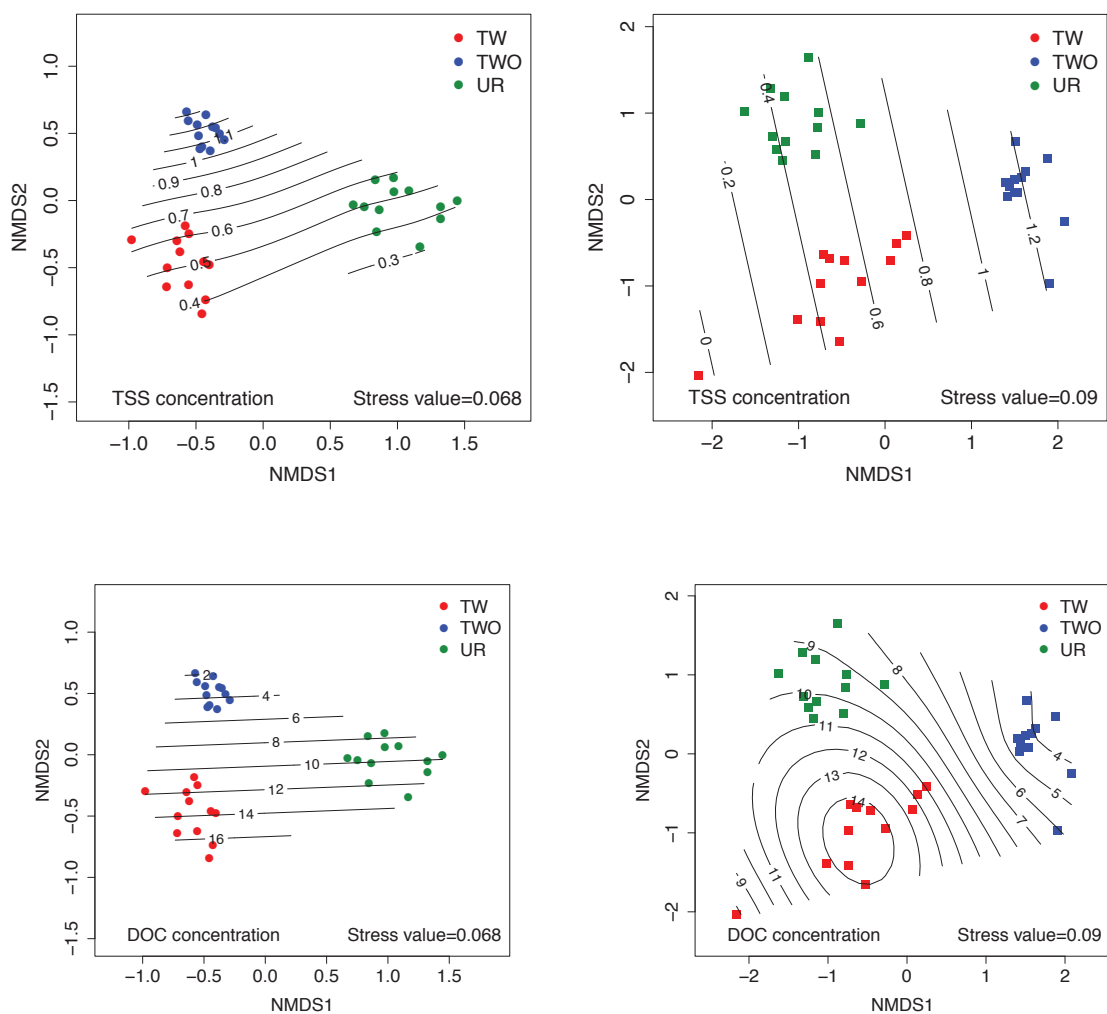


Fig 4.4: NMDS analysis based on Bray-Curtis dissimilarity of community structure in runoff water samples from different land use: teak plantation with understory (TW), teak without understory (TWO), upland rice (UR) for Particle-attached (PA) (Left side) and Free-living (FL) fraction (Right side) fraction. Gradient of dissolved organic carbon (DOC) and total suspended sediment (TSS) concentration was fit with community structure data

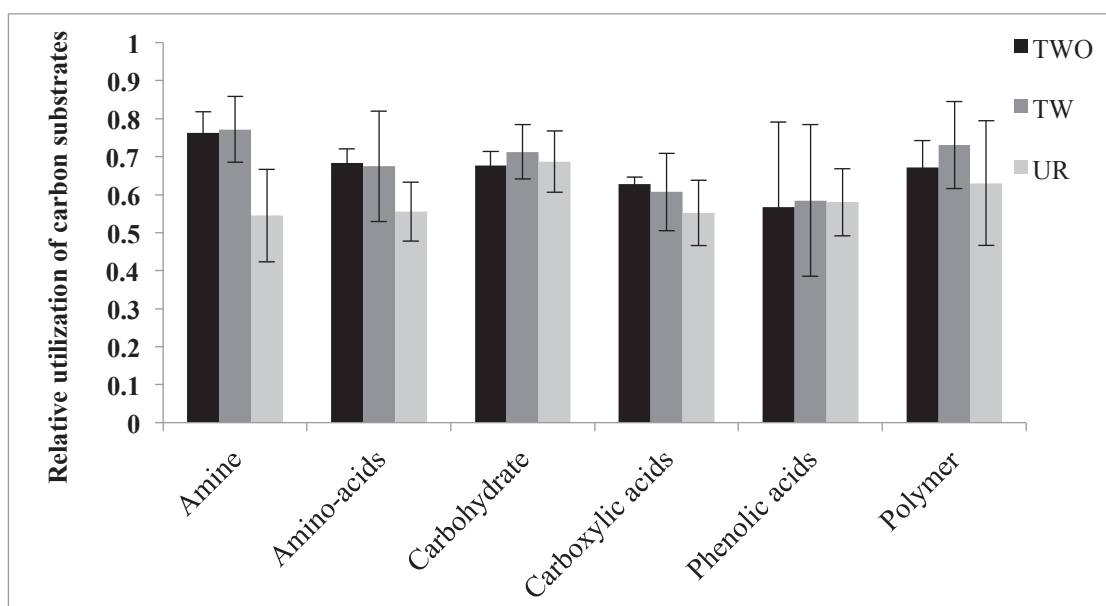


Fig 4.5: Relative utilization of carbon substrates in runoff water of composite sample (CS) under different LU.

Common OTUs between soil samples and runoff water samples

The overlap between soil samples under different LU and the corresponding runoff water for all time points together was identified during the rain simulation for both PA and FL fractions (Table 4.3). We found that the number of shared OTUs between soil samples and runoff water for each LU during the rain simulation was highest for TWO, followed by TW and UR for both the PA and FL fractions. The number of shared OTUs between soil samples and runoff water samples for PA was higher than that for FL for each LU, except for TWO. More precisely, the number of shared OTUs between soil samples and runoff water samples from TWO was $2,158 \pm 306$ and $2,359 \pm 263$ OTUs (27.1% and 29.5% of total number species in soil samples of TWO) for PA and FL, respectively. Meanwhile, the number of shared OTU between soil samples and runoff water samples from TW was 1939 ± 305 and 1109 ± 332 OTUs (22.8 % and 13 % of total number OTUs in soil samples of TW) for PA and FL, respectively. The number of shared OTUs between soil samples and runoff samples from UR was lowest amongst different soils with 1475 ± 311 for PA and 592 ± 231 for FL (17.3 % and 7 % of total number of OTUs in soil samples of UR) (Table 4.3).

Table 4.3: OTU similarity between soil and runoff water under different land use for particle-attached (PA) and free-living (FL) fractions separately during the rain simulation.

	Number of OTU in runoff water	Number of OTUs in soil	Number of shared OTU
TWO-PA	7798 ± 1191	7979	2158 ± 306
TW-PA	7123 ± 838	8482	1939 ± 305
UR-PA	6229 ± 217	8526	1475 ± 311

TWO-FL	8236 ± 965	7979	2359 ± 263
TW-FL	3881 ± 1853	8482	1109 ± 332
UR-FL	3666 ± 536	8526	592 ± 231

We identified the relative abundant of bacterial taxa in shared community between soil samples and runoff water under different LU. Interestingly, we found that 4 OTUs (OTU26, OTU16, OTU36 and OTU19) with a relative abundance higher than 1% in soil samples were shared between soil samples and runoff water samples from both TWO and TW and for both PA and FL fractions (Fig S 4.2). Meanwhile only OTU19 and OTU26 with a relative abundance more than 1% in soil samples of UR were shared with runoff samples from UR (Fig S 4.2). These four OTUs were identified as *Alpha-proteobacteria* belonging to the order of *Rhizobiales*. The remaining shared OTUs between soil samples and runoff samples from TWO, TW and UR consisted of 2.2 – 5.4 % OTUs (0.1% < relative abundance <1% in soil samples) and 94 - 97% OTUs with relative abundance lower than 0.1% in soil samples for both PA and FL (Fig S 4.2).

In this study, amongst the shared OTUs between soil and runoff water, the number of OTUs that only appeared in runoff from TWO or TW or UR (considered as specific OTUs) and the number of OTUs that appeared simultaneously in runoff from TW, TWO and UR (considered as common OTUs) were identified and compared in terms of the number and relative sequence abundance. We found that the number of specific OTUs was higher than common OTUs in runoff from all LU for PA fraction ($P < 0.001$), but the sequences abundance of specific OTUs was lower than common OTUs, especially for TWO. Meanwhile the number of specific OTUs for FL was higher than the common OTUs in surface runoff from TWO only but their sequence abundance was similar. In addition to, the number of specific OTU in the surface runoff from TWO was higher than from other LU for FL fraction ($P = 0.001$), but this pattern was not shown for the common OTUs ($P = 0.001$) (Fig 4.6).

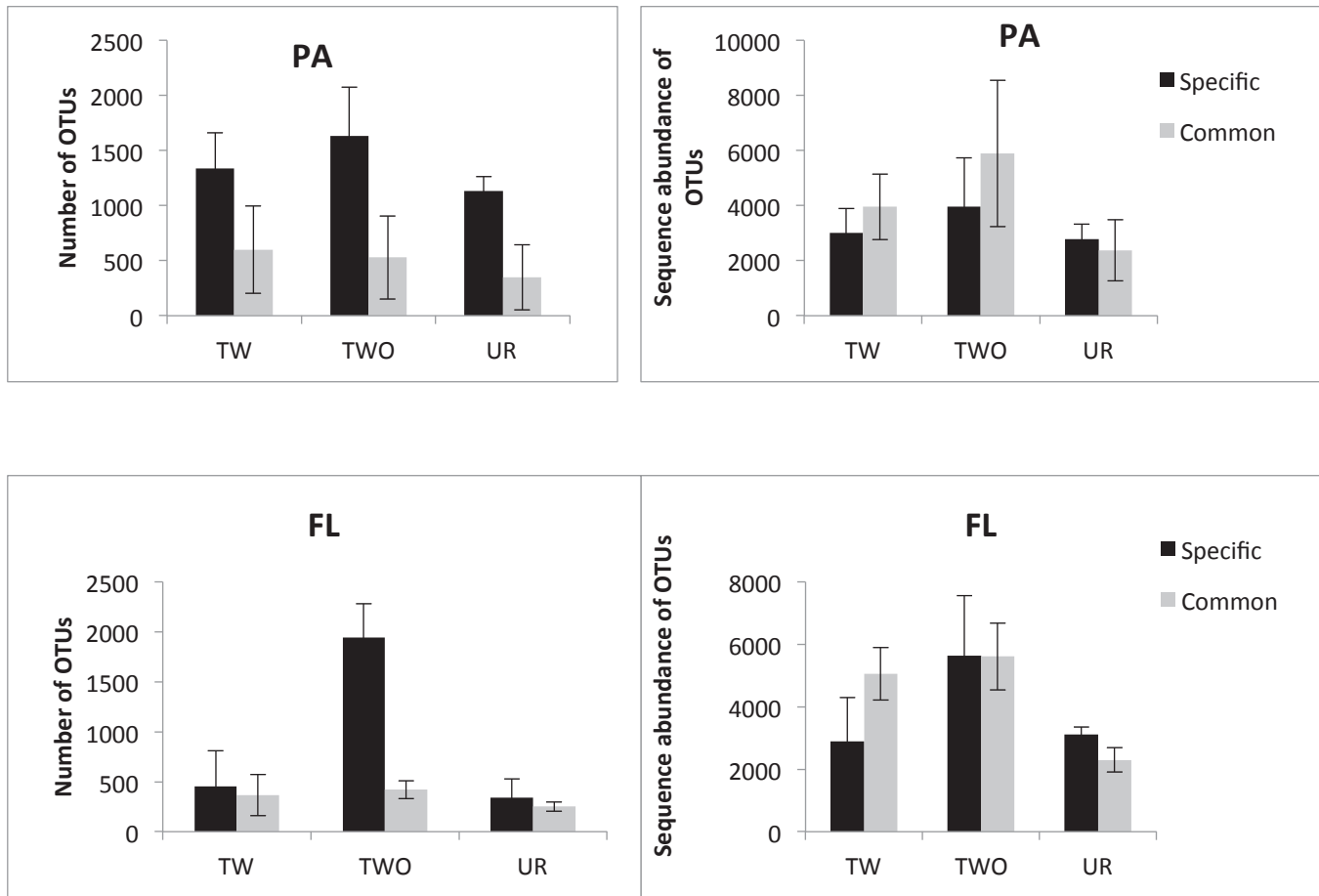


Fig 4.6: Number of shared OTUs between soil and runoff water samples from different land use: teak plantation with understory (TW), teak without understory (TWO), upland rice (UR) for Particle-attached (PA) (above) and Free-living (FL) (below) fraction. Specific OTUs classified (OTU only appeared in the runoff water from TW or TWO or UR), common OTUs (OTU appeared in runoff water from both TW, TWO and UR).

Functional groups lost from soil into runoff water

To assess the potential functional loss during a rain event, we applied FAPROTAX to identify the functional groups of all OTUs in the soil samples from each LU and for the shared OTUs between soil samples and runoff water from each LU (Louca et al., 2016). For the OTUs in soil samples, about 16% of the OTUs were assigned to at least one functional group. For the shared OTUs between soil and runoff samples under different LU, only 6-12% of the OTUs were assigned to at least one group. Despite this low identification coverage, the two most abundant functional groups in both soil and runoff water were Chemoheterotrophy and Aerobic Chemoheterotrophy. Groups belonging to specific functions relating to the nitrogen cycle such as nitrification, aerobic nitrite oxidation, aerobic ammonia oxidation was also present in all soil samples. These functional groups were also a highly abundant group in runoff water from UR (0.2 – 4 % of total abundance of functional groups) and TW (0.2 – 2 % of total abundance of functional groups), but were most abundant in the runoff water under TWO for both PA and FL (2 – 10 % of total abundance of functional groups) (Fig 4.7). The functional groups of sulfur respiration and respiration of sulfur compounds were more abundant in the runoff waters from TW and TWO for PA than in the runoff water from UR. Finally, the functional group for nitrate reduction and respiration was more abundant in runoff water from UR than in runoff from TWO and TW (Fig 4.7).

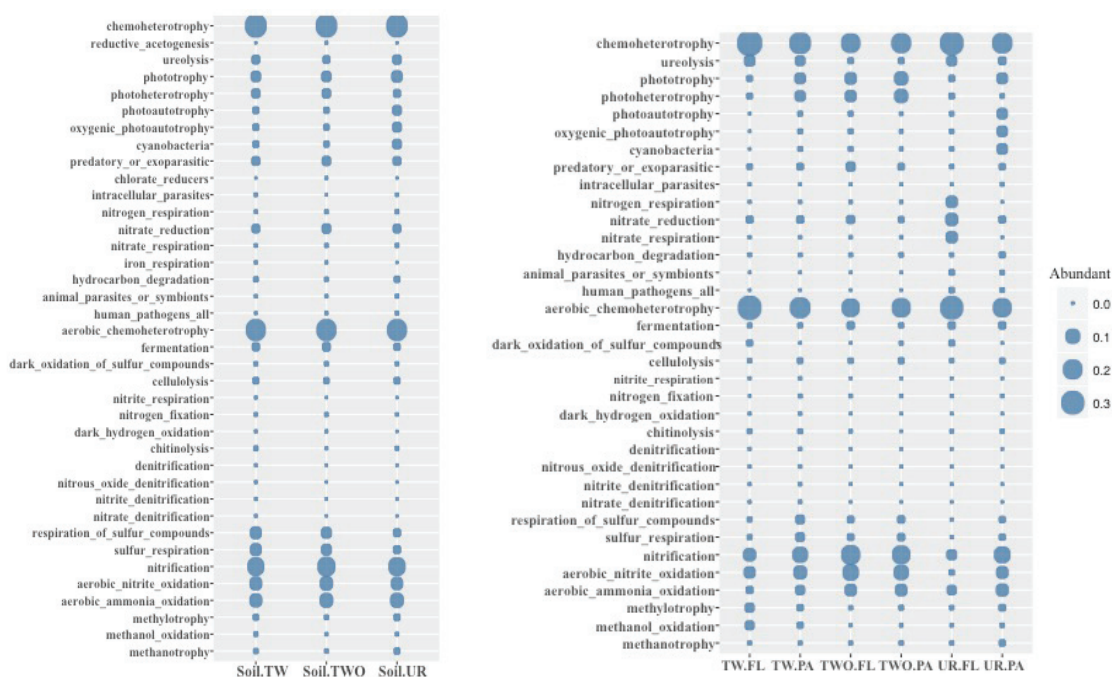


Fig 4.7: Functional groups of all OTUs in soil samples (left side), and common OTUs between soil and runoff water samples (right side) from different land use: teak plantation with understory (TW), teak without understory (TWO), upland rice (UR) for Particle-attached (PA) and Free-living (FL) fractions.

4.4. Discussion

Importance of LU in driving the quality and the quantity of DOC lost from soil into runoff during a typical monsoon rain event.

LU change from annual crops to teak plantations has occurred rapidly in the Houay Pano catchment. From 2008 to 2015, the catchment was characterized by the expansion of teak plantations and the decrease of annual crops. Most teak plantations are of more than 3 years old and farmers frequently burn the understory vegetation cover resulting in bare soils that are highly susceptible to erosion relative to other LUs (Ribolzi et al., 2017).

Our results confirmed that DOC loss from soils is highly driven by the type of LU found in the studied area. Axel et al. (2011) reviewed 385 studies on effect of LU change on SOC in the tropical economics indicated that the conversion of primary forest into annual crops (i.e. maize, bean) and perennial (i.e. sugar cane, coffee plantation) reduced 25% and 30% of SOC respectively. Erosion that is controlled by LU and land management may be a major pathway of SOC loss for tropical regions (van Noordwijk et al., 1997). Moreover, DOC concentration was high during the first minutes of runoff and decreased with time and with increasing runoff volume. This dynamic was also observed in other studies (Strohmeier et al., 2013; Janeau et al., 2014) and is most likely due to the dilution of soluble fractions in the soil surface. We observed relatively high concentrations of DOC in runoff from TW and UR (13.4 and 9.6 mg C L⁻¹ respectively) as compared to runoff from TWO (3.8 mg C L⁻¹) (Table 4.2). These values are in contrast to those of Janeau et al. (2014) who observed average DOC concentrations in runoff samples of 4.1 and 6.4 mg C L⁻¹ from the soil surface of planted forests with litter and without litter, respectively. However, the data of Janeau et al. (2014) was collected from an *Acacia mangium* forest from which the leaf litter was physically removed immediately prior to the rain simulation, rather than by annual burning.

The quantity and quality of DOC exported from soil into runoff is, in part, due to the nature of SOC inputs from vegetation and land cover (Yamashita et al., 2011). The low DOC concentrations in runoff from TWO are probably a consequence of the poor soil organic carbon concentrations as compared to TW and UR. This also corresponds with the low amount of total organic carbon in TSS in runoff from TWO. It may be due to the fact that there is little organic input from root exudation and vegetation debris in the TWO soils as all the vegetation is burnt. In addition, during burning, nutrients and carbon can be lost in the atmosphere through volatilization (gaseous form) and particulate transport (solid form). While particulates may be re-deposited on the burned site or in adjacent areas, either as dry fallout or rainfall, non-particulate losses through volatilization are more likely to represent permanent losses from the site (Wanthongchai et al., 2008).

Changes in the characteristics of DOC exported into streams as a consequence of LU change and agricultural practices have been well documented (Wilson and Xenopoulos, 2008; Williams et al., 2010).

In our study, we observed a positive correlation between DOC and humic-like components (C1 and C2), HIX and negative correlation between DOC and Sr in runoff water, as has been previously observed in the other studies on tropical streams (Wu et al., 2007) and rivers (Yamashita et al., 2010). Inamdar et al. (2011b) indicated that the decrease in DOC concentration, aromatic and humic-like constituents may be due to sorption onto mineral soil surface. The other studies also suggested that DOC with a low humic-like and aromatic content is more bio-available (Kaushal and Lewis, 2005; Maie et al., 2006). Therefore, our study suggested that DOC in runoff water under TWO had a lower molecular weight, was less aromaticity and thus was more bio-available. This also suggested that the lower DOC concentration found in TWO because it was degraded by bacterial taxa.

The loss of bacterial community diversity from soil into runoff water

These abiotic consequences of LU on soil erosion have drastic biological consequences on the type of organisms and related functions lost with the soil particles eroded into runoff waters. Indeed, it is assumed that microorganisms positioned in the soil surface have the same behavior as the soil particles (Tyrrel and Quinton, 2003), at least in a context of particle displacement during a surface water runoff event. The movement of bacteria in overland flow (or surface water runoff) may occur in form of bacteria attached to soil particles or as single, free-living cells (Tyrrel and Quinton, 2003; Jamieson et al., 2004). We hence observed higher rates of runoff, TSS concentrations and export of bacterial taxa for both PA and FL from soil into surface runoff samples under TWO as compared to TW and UR soils during the course of rain simulation. TWO harboring harsh surface conditions such as high percentage of structural crust and low infiltration may well explain the high runoff, soil loss and high microorganism detachment during the rain simulation experiment.

Tyrrel and Quinton (2003) also suggested the factors affecting the rate of export of microorganisms by overland flow includes the number of microorganisms at the soil surface, the protection of soil surface by biological material, the level of adhesion of bacteria into the particle surface and to each other, the kinetic energy of the rainfall and the overland flow velocity and depth. Here we show that the bacterial community structure in TWO was closer between PA and FL fractions in the runoff water, and between soil and the runoff water than it was in the other LU. Furthermore, although we observed a clear separation between PA and FL in runoff water under TW and UR, the PA fraction was more similar to the bacterial community in the soil sample than in FL fraction. The partitioning of different bacterial taxa in the PA and FL fraction during transport into surface runoff depends on interactions between different environmental, such as soil hydraulics including water viscosity, solutes in the soil, etc., and vegetative factors (Olilo et al., 2016). The function of the surface vegetation can decrease the loss of bacterial taxa by decreasing overland flow velocity, adsorbing bacteria onto leaves, stems and litter or onto clayey-loam soil particles or through improving percolation of bacteria entrained in runoff into the root zone systems (Olilo et al., 2016).

Burning the understorey under teak plantation in our study site removes the layer of surface vegetation upon which many taxa may live. This loss also weakens the adsorption of bacteria into soil particles and increases the possibility of taxa attached to soil particles being either eroded or detached from particles and transported as free cells. Therefore, the high soil erosion that occurs in TWO may also lead to the simultaneous wash out a large part of taxa of both PA and FL fractions during rain events.

Conversely, under UR where the intensity of soil erosion was lower than in the teak plantation, a smaller proportion of bacteria taxa were lost in runoff. Moreover, more diverse PA was exported from soil into the runoff under TW and UR than FL taxa, suggesting bacterial community was less detached by force of raindrops (FL taxa) in these LU. Thus, the main form of taxa loss in the surface runoff was taxa attached to soil particles when the rain falls. The understory below a teak plantation may provide a good surface layer protection to limit erosion of soil particles and filter the bacterial community entrained in runoff water along with the plant debris.

The positive relationship between SOC concentration and the diversity and structure of the bacterial community in soils and sediments has also been observed in many studies (Juan et al., 2015; Wang et al., 2016). SOC is known to be the main factor influencing bacterial community structure (Fierer et al., 2007a; Tian et al., 2017). In our study, we also found that the significant separation of the structure of microbial community followed different LU was controlled by DOC and TSS for both the PA and FL fractions in the runoff water. However, PA bacterial structure was more linked to DOC concentration than FL fraction. This suggests that the eroded soil particles simultaneously carry organic carbon and bacterial taxa absorbed on to it in the surface runoff. Olilo et al. (2016) also found observed that the transport *E. coli* and organic carbon in overland flow was similar.

Because a large proportion of OTUs (75-79%) in soil samples were defined as rare taxa (relative abundance less than 0.1%), most OTUs exported from soil samples into runoff water for each LU were also rare OTUs in both PA and FL fractions of the runoff waters. Crump et al. (2012) also found that 98% taxa in the soil were from rare fractions that comprised a large percentage of the total DNA sequences. Rare species are considered to be susceptible species to local environmental or biological processes (Pedrós-Alió, 2007, 2012). They were also considered to be more highly influenced by dispersal mechanisms in soils (Jiao et al., 2017). Pedrós-Alió (2012) suggested that when bacteria are transported between different environments, their concentration may decrease both by simple dilution and by the progressive death of the cells, thus forming part of the rare biosphere. This is consistency with the result found higher number of specific OTUs that had low abundance exported from each LU into the surface runoff compared to the number of common OTUs that found as high abundance for PA fraction. Moreover, in our study, the number specific OTUs that only appeared in the runoff water from TWO was found highest compared to other LU for both PA and FL. This suggests that specific bacterial community groups in TWO were more easily exported in runoff water during rain events. However, Pandit et al. (2009) indicated that specific taxa were affected by local environmental conditions whereas common

taxa were mainly governed by dispersal processes. In other words, common taxa may better adapt to a new environment, in a stream for example, as compared to specific taxa.

The loss of soil functions into runoff water

In general, the carbon metabolic capacity of the bacterial community was higher in runoff water from TW/TWO than from UR. This corresponded with the higher diversity found in the runoff water from the teak plantations, especially in TWO. This, in turn, suggests that more bacterial groups relating to function of organic carbon degradation in soils were lost in the runoff water from teak plantation as compared to UR.

Although only a small proportion of the OTUs lost from soil to runoff water were assigned to functional groups by FAPROTAX (Louca et al., 2016), our results revealed that the functional groups relating to the nitrogen cycle such as nitrification, aerobic nitrite oxidation, aerobic ammonia oxidation that were abundant in all soil samples, were highest in the runoff water from TWO. This suggests that the bacterial groups with these functions were more susceptible to loss from soils under TWO. This is particularly important because these functions are often acknowledged to be associated to rare microbes in soils (Prosser and Nicol, 2012). Moreover, we found that only 4 similar abundant OTUs (relative abundance more than 1%) were lost from soil into the runoff water under TW and TWO. All are classified as being from the *Rhizobiales* order. Groups of *Rhizobiales* are well-studied associates of plants that commonly exert beneficial functions for their hosts by providing various nutrients for essential plant metabolites (Ivanova et al., 2000). The order contains many genera of nitrogen fixing, methanotrophic, legume-nodulating, microsymbiotic bacteria (Jourand et al., 2004; Erlacher et al., 2015). Therefore, the loss of these groups from soil via surface runoff may have an adverse effect on soil productivity and function. In other words, burning understory under teak plantation (TWO) leads to more degradation of soil functions than does other LU during erosive rain events.

4.5. Conclusion

To our knowledge, this work presents the first study to simultaneously evaluate how LU drives the loss of DOC, in terms of concentration and optical characteristics, as well as related loss of bacterial community, in terms of diversity and associated functions of different fractions, during a simulated rain event. Our study found that unsustainable agricultural practices with burning understory under teak plantation cause high runoff and sediment loss, increasing the export of the bacterial community from soils into runoff water for both the PA and FL fractions, with potential strong consequences on the resulting soil functioning in middle and long-term perspectives. Hence, our results also suggested that nitrogen cycle and supporting functions of plant productivity (organic carbon degradation) were more degraded under teak plantation with burning understory.

Moreover, the loss of specific bacterial taxa from soil can affect not only soil productivity, but also downstream ecosystems. This is especially important as some eroded taxa were affiliated as members of the *Clostridium* family which includes pathogenic strains found in animal waste (Ellis and McCalla, 1976). However, it should be noted that any risk posed by a pathogen to a downstream water resource is highly dependent on its transport and survival in the catchment as well as the exposure to that pathogen.

This work also highlights the importance of LU and sustainable agricultural practices to limit land degradation relating to loss soil organic carbon, bacterial community and the associated soil functions in the tropical catchment of Southeast Asia that are suffering from disturbance and conversion from agricultural uses to planted forest.

Supplementary information

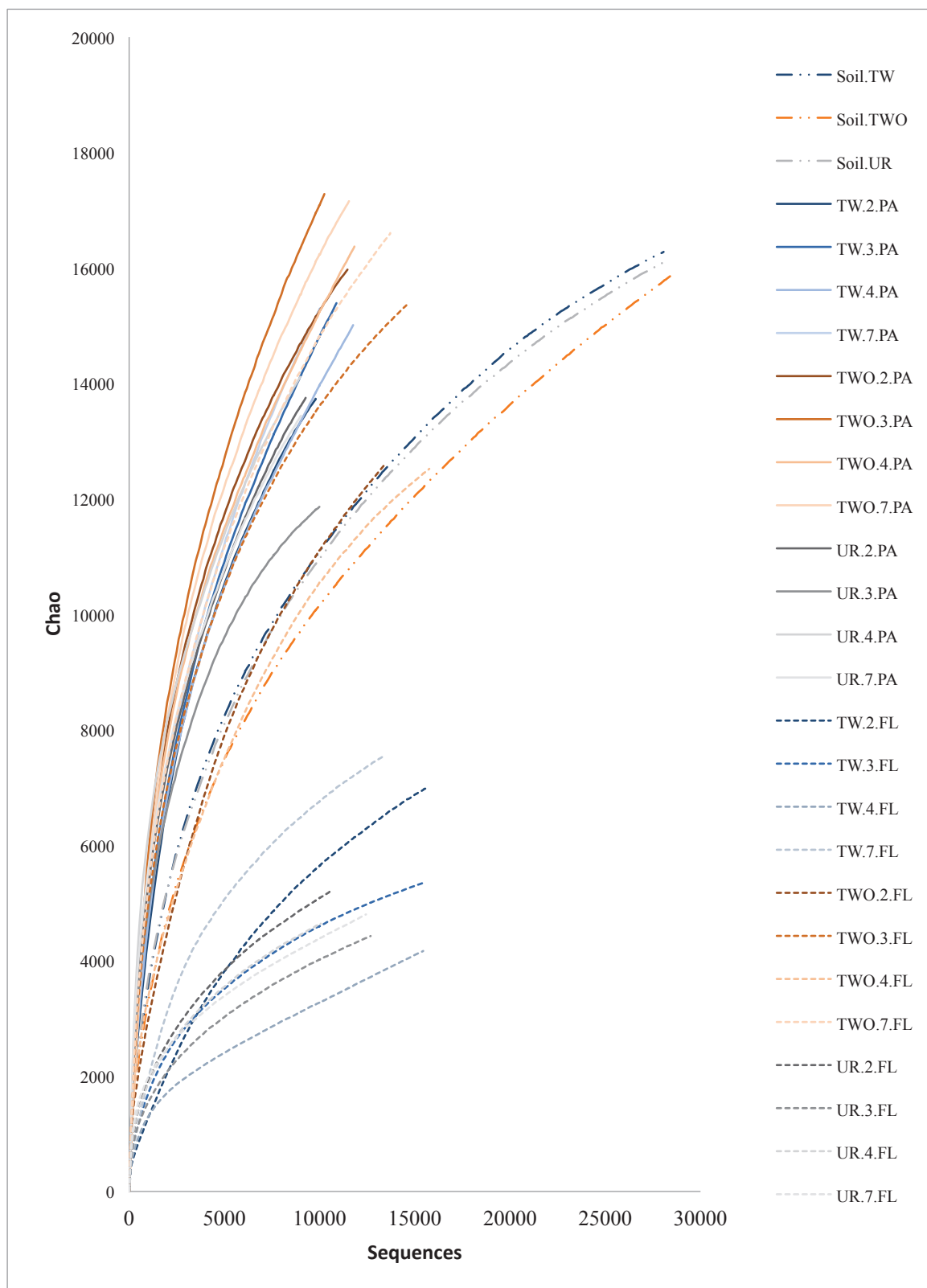


Fig S 4.1: Rarefaction curves for all samples

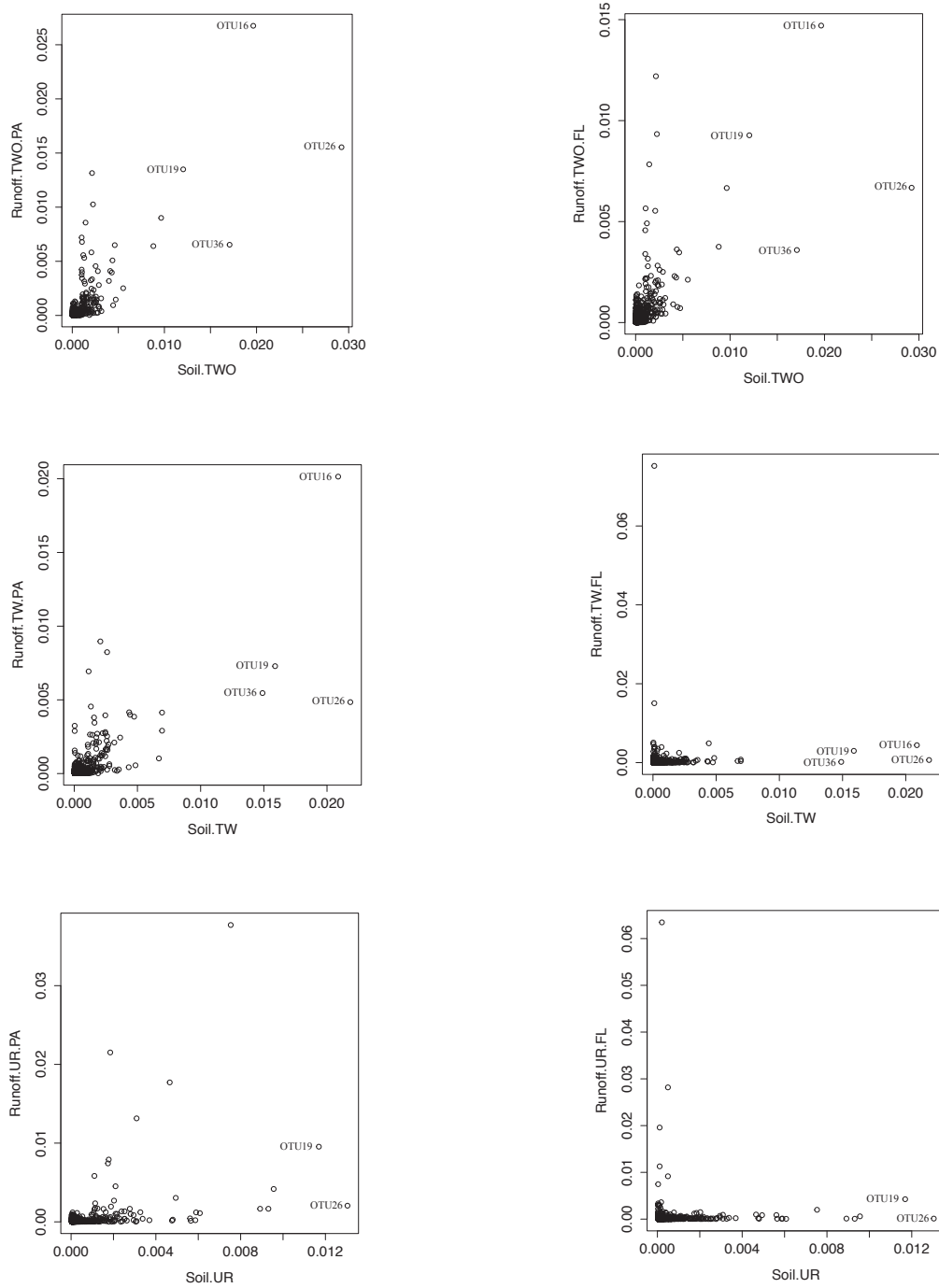


Fig S 4.2: The relative abundance of shared OTUs in soil samples and surface runoff samples under different LU for particles attached (PA) and free-living (FL) fractions. The OTUs with relative abundance more than 1% was labeled in the graph.

Table S 4.1: The richness and diversity in soil samples and runoff samples from different LUs (TW: teak plantation with understory, TWO: teak plantation without understory, UR: upland rice)

	Chao	Invsimpson	Shannon
Soil.TW	10606	477.06	7.57
Soil.TWO	9713	405.05	7.48
Soil.UR	10488	756.60	7.67
TW.1.Att	13322	943.59	7.85
TW.2.Att	14132	791.39	7.83
TW.3.Att	13330	707.77	7.82
TW.CS.Att	14608	711.78	7.87
TWO.1.Att	14687	563.82	7.81
TWO.2.Att	16343	391.35	7.68
TWO.3.Att	14578	334.41	7.55
TWO.CS.Att	15563	367.84	7.61
UR.1.Att	13608	596.98	7.90
UR.2.Att	11544	349.93	7.69
UR.3.Att	14115	298.93	7.79
UR.CS.Att	13422	212.20	7.64
TW.1.FL	5329	55.63	6.14
TW.2.FL	4413	118.08	6.68
TW.3.FL	3107	218.06	6.51
TW.CS.FL	6485	168.34	6.72
TWO.1.FL	10614	861.68	7.59
TWO.2.FL	13125	669.92	7.83
TWO.3.FL	10039	870.80	7.67
TWO.CS.FL	14197	746.59	7.88
UR.1.FL	4880	181.16	6.82
UR.2.FL	3854	126.98	6.55
UR.3.FL	4451	109.45	6.69
UR.CS.FL	4211	121.24	6.80

Table S 4.2: Result of *envfit* to test for significant differences between environmental factors and bacterial community structure. P-value (*=0.01; **=0.001; ***=0)

	NMDS1	NMDS2	R ²	P value
Particles Attached (PA)				
DOC	0.05	-0.99	0.56	0.001**
TSS	-0.34	0.94	0.4	0.001**
Free-living (FL)				
DOC	-0.75	-0.65	0.32	0.002*
TSS	0.97	0.21	0.5	0.001**

In chapter 4, I showed how LU change from upland rice to teak plantation caused higher losses of soil organic carbon and bacterial diversity, especially when understorey under teak plantation was burnt. Moreover, loss of soil functional group relating to organic carbon degradation and nitrogen cycles was higher under TWO than other LU. These results emphasized the effect of LU change on soil degradation through runoff water. Moreover, this upland runoff under current LU change and its legacy may add different SOC and soil bacterial community, which consequently may change the bacterial community of this catchment for long-term.

In the next chapter (Chapter 5) I propose to assess the general effects of LU change and its legacy on stream DOC and bacterial community in two different situations. First (5.1), these effects are considered at base and high flow by sampling the water stream at two opposite seasons (dry and wet). In a second section (5.2), I propose to survey these effects on a shorter time scale, but much higher flow rate, i.e. during a flood event that occurred in May 2015.

CHAPTER 5

EFFECT OF LAND USE CHANGE ON AQUATIC BACTERIAL DIVERSITY AND FUNCTION IN *IN SITU* INVESTIGATION SCALE

5.1. Vicinal land use change strongly drives stream bacterial community in a tropical montane catchment

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Abstract

Impact of land use (LU) change on stream environmental conditions and the inhabiting bacterial community remains rarely investigated, especially in tropical montane catchments. We examined the effects of LU change and its legacy along a tropical stream by comparing seasonal patterns of dissolved organic carbon (DOC) / colored dissolved organic matter (CDOM) in relation to variations in structure, diversity and metabolic capacities of particle-attached (PA) and free-living (FL) bacterial communities. We hypothesized that despite seasonal differences, hydrological flows that accumulate allochthonous carbon along the catchment are a major controlling factor of the bacterial community. Surprisingly, local environmental conditions that were largely related to nearby LU and the legacy of LU change were more important for stream bacterial diversity than hydrological connectivity. DOC was strongly correlated with PA richness and diversity. The legacy of LU change between teak plantation and annual crops induced high DOC and high diversity and richness of PA in the adjacent waters, while banana plantations were associated with high diversity of FL. The community structures of both PA and FL differed significantly between seasons. Our results highlight the importance of vicinal LU change and its legacy on aquatic bacterial communities in mixed used tropical watersheds.

Keywords: LU legacy, DOC, CDOM, Bacterial diversity, Land management, Hyporheic zone

5.1.1. Introduction

Although rarely the main focus of studies on aquatic microbial communities, land use (LU) change represents one of the most significant factors controlling stream diversity (Zeglin, 2015). LU change is an anthropogenic activity referring to any transition from one LU category e.g. annual crops/fallow into another category e.g. agricultural lands, planted forests or urban areas. LU change can therefore have local and more wide ranging impacts on a variety of biological, chemical and physical processes.

In catchments, LU change can affect downstream ecosystems due to the interactive links between soils and streams played by hydrological processes such as stream-groundwater interactions through the hyporheic zone (HZ) and overland flow processes (Febria et al., 2012; Ward, 2016; Lacombe et al., 2017). In streams at base flow, water follows a complex path alternating between free flow along the surface of the stream bed and sub-surface transient storage throughout the streambed sediments of the HZ (Bencala and Walters, 1983). LU change can induce changes in soil organic carbon, *i.e.* loss of soil organic carbon caused by conversion of primary forest into cropland (Axel et al., 2011) or release of the recalcitrant soil organic carbon fraction due to conversion of peatlands to paddies (Wang et al., 2017c). These changes drive the composition of the soil microbial community (Tian et al., 2017) and strongly impact catabolic responses of microbial communities (Mazzetto et al., 2016). In aquatic environments, LU change is also acknowledged to strongly modify hydrological processes – *i.e.* evapotranspiration rates (Zhang et al., 2013), runoff and flood peak discharge (Bahram et al., 2007; Zare et al., 2016), detachment rate (Lacombe et al., 2017), overland flow and sediment yields (Ribolzi et al., 2017). Recent work has shown that complex along-stream water paths through transient storage zones may influence microbial community composition at base flow (Kim et al., 2017). Therefore, aquatic community structure might be driven by the local streambed sediment composition and characteristics (carbon content, texture, permeability, etc.) which vary spatially and temporally along the stream as a function of surrounding soil sources (Evrard et al., 2016). Consequently, LU change plays an important role in the transport along streams of dissolved organic matter (DOM) (Wilson and Xenopoulos, 2008; Williams et al., 2010) as well as impacting the organisms in the aquatic ecosystem, such as planktonic microbes (Kamjunke et al., 2015) and pathogenic bacteria (Ribolzi et al., 2015; Rochelle-Newall et al., 2015).

Bacteria play a key role in the functioning of freshwater ecosystems (Allison and Martiny, 2008). Aquatic microbial communities may be affected by spatial and temporal variations in hydrological processes or in the type and degree of environmental change. In particular, seasonal shifts in water temperature and river flow can influence the diversity of temperate river bacterial communities (Crump et al., 2003). Moreover, hydrological conditions have a differential impact on particle-attached (PA) and free-living (FL) bacteria (Luef et al., 2007). Whereas the abundance of PA bacteria is influenced by the quality of particle matter imported from different flow paths, the abundance of FL bacteria is more strongly impacted by flow connectivity (Luef et al., 2007).

Changes in bacterial community composition are also linked to both autochthonous processes (e.g. phytoplankton blooms) and allochthonous factors (inputs of terrestrially derived organic matter), both of which can induce shifts in bacterial community structure (Crump et al., 2003; Allgaier and Grossart, 2006). Some studies have indicated that the sources and characteristics of DOC could drive aquatic bacterial community functioning. For example, in temperate streams, allochthonous DOM from agricultural activities has lower molecular weight compounds that were more labile to microbial community than DOM from forest and wetlands (Williams et al., 2010). Similarly, fresh humic DOC originating from forest headwaters supported anabolism at a higher level of bacterial growth efficiency in adjacent rivers than peat harboring pre-processed humic DOC (Berggren and del Giorgio, 2015).

As part of the bulk DOM pool, chromophoric (or coloured) dissolved organic matter (CDOM) can be used a proxy for changes in the chemical characteristics of the DOM pool (Fellman et al., 2010; Yamashita et al., 2011; Ishii and Boyer, 2012). Several indices, based on the absorption or fluorescence at specific wavelengths have been proposed. For example, the absorption at 254nm divided by DOC concentration, also known as the $SUVA_{254}$, provides a proxy for aromaticity (Weishaar et al., 2003). The spectral slopes (S), determined over selected wavelength ranges such as between 275-295 nm or between 355-400 nm are surrogates for average molecular size and bioavailability and hence can provide information on the sources and diagenetic state of the DOM (Helms et al., 2008). The spectral slope ratio, S_r , determined as $S_{275-295} / S_{355-400}$, provides information on the apparent molecular weight of the DOM (Helms et al., 2008; Fasching et al., 2016). These indices can also be viewed as proxies for bioavailability. $SUVA_{254}$ is negatively correlated with biodegradable DOC (Fellman et al., 2008) and positively correlated with the hydrophobic organic acid fraction of DOM (Spencer et al., 2012), while higher S values indicate low molecular weight material and decreasing aromaticity and, hence, potentially increased bioavailability for bacteria (Fichot and Benner, 2012). Fluorescence three-dimensional excitation-emission matrices (EEMs) of CDOM can also provide information on DOM source and on its bioavailability (Fellman et al., 2010). For example, EEMs have been used to study the effects of LU on stream biogeochemistry (Wilson and Xenopoulos, 2009) or to follow DOM bioavailability in streams (Wickland et al., 2007; Fellman et al., 2009).

Yet, to our knowledge, there is no integrated understanding of how current and past LU change (or the legacy of LU) via hydrological processes, in relation to DOC, impacts PA and FL stream bacteria communities, especially in the tropical regions. In Southeast Asia, LU change such as the replacement of annual crops with tree plantations in upland catchment areas is increasing rapidly (Ribolzi et al., 2017). Moreover, these ecosystems have specific geographical, pedological and meteorological characteristics (high volume, intense rainfall events, high slope, and low soil organic carbon contents). The combination of LU change with these specific environmental factors results in high rates of soil erosion, a process known to be predominantly driven by LU change rather than environmental characteristics at the catchment scale (Valentin et al., 2008).

Here we investigated how LU change and its legacy impacted the concentration and characteristics of DOC and CDOM in the stream, and how these two factors subsequently drove the dynamics of aquatic bacterial metabolic capacity and diversity along the Houay Pano stream, Laos. This archetypal mixed LU catchment harbors typical seasonal variations such as found in the tropics. In the past two decades, it has experienced rapid LU change from upland crops (*e.g.* upland rice) with long fallow periods to annual crops (*e.g.* job's tears, maize) or forests (*e.g.* teak and bananas plantations) with reduced fallows and traditional slash and burn management techniques that removed the presence of a viable understory (Patin et al., 2012). The loss of the understory led to patches of bare soil with increased soil crusting rates and decreased soil permeability resulting in increased overland flows and soil losses (Ribolzi et al., 2017). The recurrence of annual crops without any fallow or rotation had a strong negative impact on soil fertility at the catchment scale (Valentin et al., 2008). It can therefore be hypothesized that the legacy of such LU change strongly modified stream microbial metabolic capacity and diversity, as a consequence of disrupted recycling of dissolved organic matter. As the streams in such steep catchments drain relatively large spatial areas under diverse LU practices, we hypothesized that despite seasonal differences, hydrological flow that accumulates allochthonous carbon along the catchment was a major controlling factor of the stream bacterial community structure.

5.1.2. Materials and Methods

Study site

The study region is located in the Houay Pano catchment area (19°51'N–102°10'E), 10 km South-West of Luang Prabang city, Laos (Fig 5.1). The regional climate is classified as monsoon tropical with two seasons: a wet season from April to September and a dry season from October to March. The average annual precipitation is 1585 mm year⁻¹, more than 80% of which occurs during the wet season (Ribolzi et al., 2008).

Five sites along the river were sampled (Table 5.1). Around each sampling site, concentric drainage areas with radius 50, 100, 150, 200 and 250 m were constructed from the TanDEM-X Digital Elevation Model (Fig S 5.1). Each drainage area is cumulative i.e. it includes the smaller areas. This spatial structure is used to analyze soil type, LU and its changes.

Table 5.1: Description of UTM coordinates and land use characteristics around sampling sites

Sampling sites	UTM coordinates (WGS84)		Land use characteristics
	Easting	Northing	
RIB72	204158.163	2198513.314	Upmost site of the catchment, surrounded mostly by fallow and teak plantations
S1	203896.417	2198512.136	First gauging stations on the stream, surrounded by bananas and teak plantations as well as rotating lands
RIB48	203670.637	2198364.471	Typical upland bog that is surrounded with teak plantation
S4	203501.245	2197660.376	Second gauging station of the stream that is surrounded with teak plantations
S9	203597	2197416	Outlet of the village Lak Sip

LU change and its legacy

The Houay Pano catchment is characteristic in that it is experiencing the rapid changes in LU occurring over Southeast Asia. This catchment has been monitored since 1998 and during this time LU has changed from shifting cultivation with long fallows to planted forests, notably teak plantations. The area occupied by teak plantations has increased from 20% in 2008 to nearly 40% area in 2012 (Huon et al., 2013). Between June 2014 and March 2015, LU did not change significantly in Houay Pano and fourteen LU types were recorded (Table 5.2).

The most important LU change occurred between 2012 and 2014 when the transition from crop to teak occurred in some areas of the catchment. To track the LU change, LU classes were simplified (Table S 5.1) e.g. cultivated parcel followed with fallow the next year was not regarded as a LU change. According to this new LU typology, spatial zones were delimited as a function of the changes occurring between 2012 and 2015. Taking into account ground surveys and geo-referencing accuracies, a treatment based on a majority filter (window 5x5, pixel size 10 m) suppressed isolated pixels. Plots smaller than 350 m² were removed from the calculation (Fig S 5.2).

Table 5.2: Land use in the wet season and dry season (unit: ha)

Land use	Wet season	Dry season
Fish	0.12	0.12
Sweet potato	0.1	-
Vegetable gardent and fruit tree	0.245	0.245
Broom grass field	0.345	0.345
Maize	0.092	0.092
Job's stear	0.589	0.111
Banana	2.782	2.782
Forest (decidious)	0.832	0.832
Forest (Dipterocarpe)	4.693	4.693
Teak plantation	23.98	23.87
Upland rice	1.788	1.18
Village	1.428	1.428
Rotating land	29.55	31.16

Sample collection

Stream water samples were collected by hand in 1.5 L new, clean plastic bottles that were rinsed 3 times with sample before collection. Five sites were sampled along the stream during base flow during the wet season (12th June 2014) and the dry season (22th March 2015). Within a maximum of 2 hours after collection, the samples were returned to the laboratory for filtration. Samples were maintained at ~20-22°C (*in situ* water temperature) and in the dark pending filtration (< 2h). Immediately prior to sub-sampling, samples were vigorously shaken to limit sedimentation biases; *i.e.* to insure sampling of suspended particles.

Measurement of DOC and CDOM

A subsample of 200 mL of each sample was filtered through Whatman GF/F glass fiber filters to remove large particles. For the determination of DOC concentration, duplicate 30 ml of this filtrate was kept in pre-combusted (450°C, overnight) glass tubes, preserved with 36 µL 85% phosphoric acid (H₃PO₄) and sealed with a Teflon lined cap in order to remove inorganic carbon. Samples were stored at ambient temperature and in the dark until measurement. DOC concentration was measured on a

Shimadzu Total Organic Carbon (TOC) V_{CPH} analyzer following the method described in Rochelle-Newall et al. (2011). DOC concentration is expressed as mg of organic C per liter of stream water.

For CDOM, 100-ml filtered samples were stored in pre-clean 125-ml amber glass bottles seal with Teflon lines cap. After collection, the samples were stored frozen (-20°C) until measurement. Before the optical measurements, the samples were thawed slowly to room temperature and re-filtered at 0.2 µm (Sartorius Minisart NML Syringe filters) to remove particles. CDOM absorption was measured with a spectrophotometer (Analytica.Jena Specord 205 UV-VIS) from 200-750 nm using a 10 cm quartz cell and Milli-Q water as the blank. The absorbance values were converted to absorption coefficient, $\alpha(\lambda)$, m⁻¹, calculated as

$$2.303 \cdot A/l \quad \text{equ1}$$

Where A is the absorbance (log I₀/I), l is the path length (in m), and 2.303 converts between log₁₀ and natural log.

SUVA₂₅₄, a surrogate for DOM aromaticity, was also calculated from DOC-normalized absorption coefficient at wavelength 254 nm (a(254):DOC), expressed in units of mg⁻¹ L m⁻¹.

The dependence of $\alpha(\lambda)$ on λ is described using equ2

$$\alpha(\lambda) = \alpha(\lambda_0) e^{-S(\lambda - \lambda_0)} \quad \text{equ2}$$

Where $\alpha(\lambda)$ is the absorption coefficient at wavelength λ , $\alpha(\lambda_0)$ is the absorption coefficient at a reference wavelength, and S is the spectral slope coefficients in the $\lambda_0 - \lambda$ -nm spectral range. The spectral slopes of absorption coefficient for 275-295 nm (S₂₇₅₋₂₉₅), 350-400 nm (S₃₅₀₋₄₀₀) was estimated using a linear fit of the log-linearized a (λ) spectrum (equ2) over spectral ranges and the ratio of the two slopes (S_r) was calculated.

Excitation – Emission – Matrices (EEM) measurements were made on a Gilden Fluorosens fluorometer using a 1 cm quart cuvette with 5nm bandwidths for excitation and emission at an integration time of 100 ms. Excitation scans were made over a range of 200 to 450 nm at 5 nm increments and emission from 220 to 600 nm at 2.5 nm increments. EEMs were corrected for inner filter effects and the manufacturers' machine correction was applied. EEM fluorescence of Milli-Q water blank was subtracted from that of sample EEM.

PARAFAC analysis was carried out in MATLAB (version R2016a 9.0.0) with the DOMFluor toolbox for MATLAB (Murphy et al., 2013) in order to decompose the fluorescence signal into a series of tri-linear structures. Processing data was done to minimize the impact of scatter lines that caused some mathematical difficulties, including removal of Rayleigh and Raman scatter. EEM wavelength ranges were reduced to excitation 290-450 nm and emission 300-600 nm. PARAFAC model generated 3

components using EEMs from our samples (n=10). The three-component model (C1-C3) was validated using split-half and random initialization methods.

Metabolic capacities of bacterial communities

Biolog[®] Ecoplate was used to determine catabolic capacity of bacterial communities. These 96-well micro-plates include triplicates of 31 different carbon-based substrates and one water control. Each well also contains a tetrazolium violet dye that turns purple upon substrate oxidation (Stefanowicz, 2006). One ml water sample was diluted 10 times with 0.2 µm filtered stream water to avoid osmotic shock and allow detection of color changes. One hundred and fifty (150) µl of this diluted sample was added to each well, and the plates were then incubated in the dark at 25 – 27°C without shaking. Following a short shaking (2 secs at 100 rpm) well color development was measured at 590 nm using a portable spectrophotometer (Bio-Rad laboratories, iMark Microplate Reader) after 12 hours incubation and then every 24h for 72h. The water blank was subtracted from the carbon substrates, which were then grouped into 6 substrate families (amine, polymers, phenolic acids, carboxylic acids, carbohydrates, amino acids) (Pommier et al., 2014). Metabolic potential of each community was normalized to each substrate family relative to the highest performance in the substrate family.

Assessment of bacterial diversity

DNA extraction and 16S rRNA gene sequencing

Fifty (50) milliliters of each sample was first filtered through a 3µm pore size filter (Polycarbonate Whatman) and a 0.2µm (Polycarbonate Supor) filters to separate Particles Attached (PA) fraction (>3.0µm) from Free-living (FL) fraction (<3.0µm and >0.2µm) (Crump et al., 1999). All filters were stored at -20°C until DNA extraction that followed a modified protocol adapted from Fuhrman et al. (1988). Briefly, the filters were cut in two halves using ethanol cleaned scissors and placed in separate Eppendorf[®] tubes. One tube was placed on ice and 525 µl of lysis buffer was added to start breaking Gram negative bacterial cells. The other tube was stored at -20°C as a back-up filter. Three cycles of freeze-thaw switches (65°C for 2 min – ice for 5 min) were then performed to facilitate cell membrane breakage. Then 0.5 g of glass beads (0.5 mm) was added into the tubes. The tubes were shaken for 45 s at 6 m s⁻¹ using a Fastprep[®] (Millipore, Fastprep[®]-24, USA) and were incubated at 4°C for 5 mins; these steps were performed twice. 11 µl of Lyzozyme (1 mg mL⁻¹, final concentration) was added to the tube and left for 30 min at 37°C to break down bacterial cell wall (cell lysis). Sodium dodecyl sulfate (SDS 10%) and proteinase K (final concentration, 100 µg ml⁻¹) were added to the tubes and were incubated at 55°C for 2 hours under constant shaking conditions (180 rpm) to remove lipid membrane. A sodium chloride (NaCl 5M) and a cetyltrimethylammonium bromide (CTAB) solution (final concentration, 1% in a 0.7 M NaCl solution) was added to the tubes, mixed and incubated at 65°C for 10 min to separate DNA from protein. Nucleic acids were then extracted twice from digestion products with phenol-chloroform-isoamyl alcohol (25:24:1); the aqueous phase containing nucleic acids was kept and purified

by adding phenol-chloroform-isoamyl alcohol (25:24:1). After isopropanol (0.6 volume of tube) addition, the nucleic acids were precipitated at -20°C for 12 h. After centrifugation, the DNA pellet was rinsed with 99% pure ethanol to remove the salt previously added. The samples were spun 20 min at maximum speed (15,000 rpm) at 4°C and the supernatant was removed. The DNA pellets were dried in a Speed Vac® (Labconco, USA) for 10 min and re-suspended in 50 µl of molecular cleaned distilled water. Nucleic acid extracts were stored at -20°C and sent overnight to Molecular Research Laboratories (Texas, USA) for further PCR amplification, product cleaning, library construction and high throughput sequencing.

The PCR primers 515F (5'-AGRGTTTGATCMTGGCTCAG-3') and 806R (5'-GTNTTACNGCGGCKGCTG-3') (producing a 291bp amplicons) (Capone et al., 2011) with sample-specific barcodes on the forward primer were used to amplify the V4 variable region of the 16S rRNA gene. Thirty (30) cycles of PCR were performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 mins, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 mins was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification, the relative intensity of bands and to check the expected amplicon size (291bp). The 10 samples included in this study were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled PCR products were purified using calibrated Agencourt AMPure XP magnetic beads according to the manufacturer's guidelines. Briefly, the pooled PCR products were incubated with magnetic beads (1.8:1 ratio of beads to sample) that pull down the PCR product to the bottom of a tube for subsequent washing steps. The clean PCR product is then release from the magnetic beads for elution. The pooled and purified PCR product was then used to prepare a DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing was performed at MR DNA (www.mrdnalab.com, Shallowater, TX, USA) on an Illumina MiSeq machine following the manufacturer's guidelines.

Sequences processing and data analysis

The MOTHUR software (v. 1.33) (Schloss et al., 2009) was used to process 16S rRNA gene sequence reads following the SOP. From the original 632,196 and 1,313,266 reads for PA and FL respectively, short reads (<250 bp) and reads with ambiguous primer or barcode sequences were discarded. Corresponding reads were paired in single sequences with an average length of 486 bp. Sequencing errors were reduced by aligning remaining reads to the SILVA database (Pruesse et al., 2007), screening the alignment to the overlapping region, and pre-clustering sequences distant by < 2bp. Chimeric sequences were identified using the integrated version of UChime (Edgar et al., 2011) and removed accordingly. To avoid misinterpretation, sequences that were classified as "Chloroplast", "Mitochondria", or "unknown" lineages were removed before clustering into Operational Taxonomic

Units (OTUs). The remaining 494,724 and 1,023,897 sequences for PA and FL respectively were clustered in OTUs with a pairwise distance < 0.03 substitutions per nucleotide with average neighbor method and considered for further analyses. Taxonomic assignments were performed on the alignment of consensus sequences with the RDP database (Cole et al., 2005). After this streaming process, 90,858 and 115,573 OTUs were found for PA and FL respectively from which 18,359 sequences were randomly subsampled for each site to perform community comparisons. Diversity indexes were calculated to compare richness and evenness of bacterial community between different sites. Bray-Curtis distances were also calculated to estimate the dissimilarity in structure between all samples, and represented as a dendrogram with `tree.shared` command in MOTHUR.

Accession numbers: The raw sequence files were deposited in the NCBI sequence Read Archive (SRA) under BioProject no. PRJNA416158. This study project has been deposited at DDBJ/ENA/Genbank under the accession KBTU00000000. The version described in this paper is the version KBTU01000000.

Statistics and partial least square analysis

DOC concentrations and optical indices between different sites and seasons were compared using ANOVA in R studio after having checked the normal distribution of the data and the homoscedasticity of the residuals. When necessary, the data were log-transformed to assure normality. Turkey HSD was used to test pairwise comparison between sites. Correlations between DOM concentrations and optical index, alpha diversity (*i.e.* Chao, Invsimpson, Shannon index) was estimated using the Pearson correlation coefficient with the `Hmisc` package in R (Harrell, 2018). AMOVA test was performed using MOTHUR software to determine the significance of the difference in community structure between dry and wet season for PA and FL communities. All differences were considered significant when the p-value of the tests was lower than to 0.05.

To explain the influence of environment on the bacterial fauna, the data were organized in two tables. The first table described the environmental variables obtained by physicochemical measurements or built from soil type, soil occupation and its changes within concentric drainage areas. The second table characterized the bacterial communities. To disentangle the seasonal from the land-use effects, we considered the samples taken during the dry and the wet season separately. The analysis focuses on factors explaining the typology of the stations. The typology of seasons was considered after as it mainly relies on physicochemical variables with low weight.

Two reasons justify the use of Partial Least Square (PLS) analysis to explain the relationship between bacterial diversity and environmental variables: i) the number of variables in both of the tables was much greater than the number of individuals; and ii) the low sensitivity of PLS to multicollinearity (Abdi, 2010) was adapted to our dataset which presented some redundancy, especially for data obtained from the concentric drainage areas. All factor computing between the bacterial diversity and the

environment tables was conducted using the R packages Plsdepot (Sanchez, 2012) and the graphical identification of the relevant variables was performed using ADE-4 (Thioulouse et al., 1997).

As the computing procedures produced numerous and redundant data that were hard to interpret, the PLS was applied using a two-step procedure. A first PLS was performed and the relevant variables retained based on the VIP (Variable Importance for the Projection) criterion where values of VIP must be greater than 0.8. If two variables describe the same environmental characteristic and differ only by the size of the concentric drainage areas, only the variable with the smallest size was kept. For example, as Banana builds LU variables with VIP equal to 2.24 for three concentric areas of radius 50, 100 and 150, only “Banana 50” was retained. Q_2 was also used to estimate the number of factors to be retained. A factor was retained if its Q_2 value was greater than an arbitrary value generally set to 0.0975 (Abdi, 2010). All selected variables are presented in the Supplementary information (Supplementary Tables S1, S2 and S3). A second PLS was applied with the variables retained from the first PLS to select the most important factors among the environmental variables that could potentially drive bacterial metabolic capacities and the diversity of the most abundant OTUs.

Co-occurrence Network

To identify the specific response of the OTUs to environmental variables (*i.e.* DOC/CDOM characteristics), co-occurrence network analysis was performed on 4 datasets that separated the OTUs occurring at each of the sample sites into wet and dry seasons and PA from FL fractions separately. To limit the number of correlations appearing in the network, only OTUs with relative abundances $> 0.1\%$ in at least one sample from the wet or dry season for the PA or FL fraction was retained. Randomness in co-occurrence of OTUs in the dry and wet seasons datasets was tested against a null model using the quasiswap algorithm (Miklós and Podani, 2004). Checkerboard score (C-score) metric which measures species segregation was performed on 50,000 simulations (Stone and Roberts, 1990) using the *oecosimu* command in the vegan package in R (Oksanen et al., 2007). The C-score represents the mean number of checkerboard units (CUs) per pairs of OTUs in each dataset and does not require perfect checkerboard distributions (Claire et al., 2007). The number of CUs for any species pair can be calculated as (Stone and Roberts, 1990) :

$$CU=(R_i - S)(R_j - S)$$

where R_i and R_j are the total abundances of OTU i and j , and S is the number of sites containing both species.

The co-occurrence network for each season was built based on the Spearman correlation matrix with the Hmisc package in R (Harrell, 2018). All results with high Spearman correlation coefficients (*i.e.* $R > 0.8$) and significance levels ($p < 0.05$) were used to build the network. The nodes in each network represent OTUs and the edges that connect these OTUs show correlation coefficient between OTUs. The connectivity of the network is equal to the number of edges in the network divided by the potential

number of edges if the network was fully connected (= number of nodes * (number of nodes - 1)/2). Network images were generated using Gephi v. 0.9.1 using the Fruchterman-Reingold layout algorithm (Bastian et al., 2009).

Methodological approach

Our method of 16S rRNA sequencing allows identification of the community structure and taxonomy of stream bacteria. Though having limited functional resolution and lower sensitivity, the 16S rRNA gene gives broad shifts in community diversity over time with a wide, comprehensive 16S rRNA gene database for deep taxonomy as compared to metagenomic data (Poretsky et al., 2014). In our study, 16S rRNA allowed the assignment of the bacteria community that was correlated with DOC/CDOM to genus level and the construction of a co-occurrence network.

5.1.3. Results

Legacy of LU change

In 2014 and 2015 when our sampling occurred, teak plantations covered a large proportion of the catchment surface area (approximately 23.3 ha (35%), distributed from middle to downstream sections of the catchment. Shifting cultivation with rotating LU including periods of fallow remained consequent with approximately 30 ha (46 %), mostly distributed in the upstream sections (Table 5.2). A part of the catchment was also covered by upland rice and crops such as banana, maize and Job's tears that are scattered along the stream length (Fig 5.1). Overall, LU was stable between 2014 and 2015. The only exception was a slight decrease in the surface covered by upland rice and increase of that covered by fallow areas/rotating LU in 2015 (Table 5.2). Between 2012 and 2015, only 7% of the area was affected by LU change: annual crops to teak plantations (3 ha), teak plantations to annual crops (1.5 ha) and the transition from forests into annual crops (0.5 ha) (Table S.5.2). Nevertheless, even though these changes apply to small areas, the following analyzes show their significant influence, probably because they were located near our sampling sites.

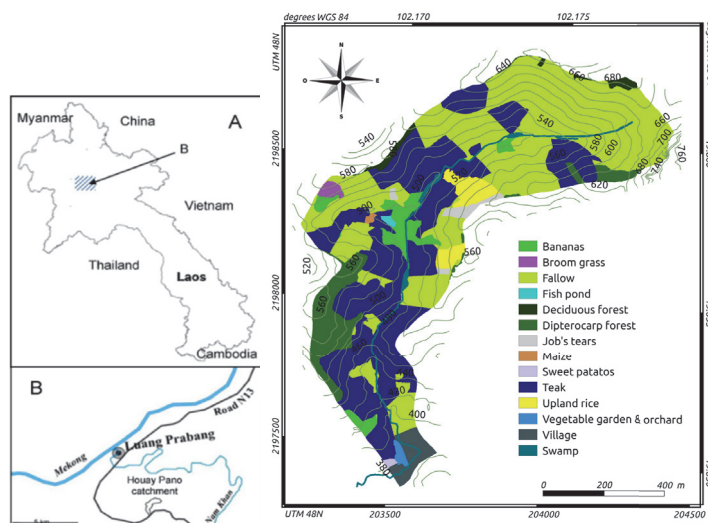


Fig 5.1: Study site: A) location of Laos in Southeast Asia, B) location of the Houay Pano catchment and land use in 2015

Variations in DOC/CDOM characteristics

DOC concentrations differed significantly between seasons (ANOVA, $P < 0.001$) in the gauging stations (S1 and S4), in the upland bog (RIB48) and notably, at the outlet of the village (S9), where DOC concentration was two times higher in the dry season. DOC concentration was highest in the upland bog (RIB48) and at the outlet of the village (S9) in the wet season ($3.2 \pm 0.06 \text{ mg L}^{-1}$ and $3.05 \pm 0.03 \text{ mg L}^{-1}$, respectively; Table 5.3). Similarly in the dry season, the sample from the outlet of the village (S9) showed the highest DOC concentration ($6.25 \pm 0.16 \text{ mg L}^{-1}$), which was six times higher than the lowest concentrations at the two uppermost sites (RIB72 and S1).

Table 5.3: Dissolved organic carbon (DOC) concentration (mg L^{-1}) and its optical indices (SUVA_{254} $\text{mg}^{-1} \text{L m}^{-1}$ and Sr) in different sites in the wet and dry season.

Season	Site	DOC (mg L^{-1})	SUVA_{254} ($\text{mg}^{-1} \text{L m}^{-1}$)	Sr
Wet season	RIB72	1.28 ± 0.04	2.61 ± 0.05	1.07 ± 0.07
	S1	1.47 ± 0.02	7.67 ± 0.06	0.57 ± 0.007
	RIB48	3.21 ± 0.06	4.60 ± 0.08	0.87 ± 0.01
	S4	2.25 ± 0.02	7.27 ± 0.07	0.83 ± 0.001
	S9	3.05 ± 0.04	5.25 ± 0.08	0.93 ± 0.01
Dry season	RIB72	1.17 ± 0.01	2.59 ± 0.01	1.1 ± 0.04

	S1	1.11±0.01	5.53±0.07	0.80±0.01
	RIB48	2.13±0.01	5.09±0.01	0.83±0.001
	S4	2.00±0.01	6.62±0.02	0.75±0.007
	S9	6.25±0.16	3.77±0.02	0.99±0.02

SUVA₂₅₄, a proxy for DOC aromaticity, ranged from 2.61 ± 0.05 to 7.67 ± 0.06 in the wet season, and 2.59 ± 0.01 to 6.62 ± 0.02 in the dry season. The highest SUVA₂₅₄ index was found at the two gauging stations S1 and S4 (7.67 ± 0.06 and 7.27 ± 0.07 , respectively) in the samples from the wet season, and at S4 (6.62 ± 0.02) in the samples from the dry season (Table 5.3). Sr, which is negatively correlated with molecular weight, ranged in the wet season from 0.57 ± 0.007 at the first gauging station (S1) to 1.07 ± 0.07 at the uppermost site (RIB72). The samples from the dry season ranged from 0.75 ± 0.007 at S4 to 1.1 ± 0.04 at the uppermost site (RIB72) (Table 5.3). SUVA₂₅₄ was negatively correlated with Sr (Correlation coefficient, $R = -0.75$, $P < 0.01$).

The PARAFAC model on 10 EEMs identified and validated three components. Component 2 (C2) presented a fluorescent peak at an excitation/emission (Ex/Em) wavelength of 300/355 nm, which is similar to Tryptophan-like fluorescence (Yamashita and Tanoue, 2003; Zhang et al., 2010). Component 1 (C1) has two peaks at an Ex/Em wavelength of 330(340)/452(467) that respectively resemble fulvic acid (Coble, 1996, 2007; Yamashita et al., 2011) and humic fluorophore group exported from agricultural sub-catchments (Stedmon and Markager, 2005). Component 3 (C3) showed a peak at an Ex/Em of 290(390)/470 that was also identical to the previously identified humic-like component (Stedmon and Markager, 2005).

While the Tryptophan-like component (C2) was positively correlated with SUVA₂₅₄ (Correlation coefficient, $R = 0.6$, $P < 0.01$) and negatively correlated with Sr (Correlation coefficient, $R = -0.6$, $P < 0.01$), fulvic acid-like (C1) and humic-like (C3) components were highly and positively correlated with each other (Correlation coefficient, $R = 0.99$, $P < 0.01$), and they also showed strong correlation with DOC concentration (Correlation coefficient, $R = 0.74-0.87$, $P < 0.01$).

Patterns of carbon metabolic capacities of bacterial communities

Except for the uppermost site (RIB72), the pattern of metabolic activity (after 72h incubation) was similar for the different samples at both seasons but did not increase from upstream to downstream (Fig 5.2). For all sites but except for RIB72, potential metabolic capacities were higher at dry than at wet season (ANOVA, $P = 0.0013$). Substrate utilization was the highest in the upland bog (RIB48), followed by the outlet of the village (S9), then the reference gauging station S4, and was the lowest at the most elevated gauging station (S1). This order was similar to the one observed for total DOC concentration in the wet season.

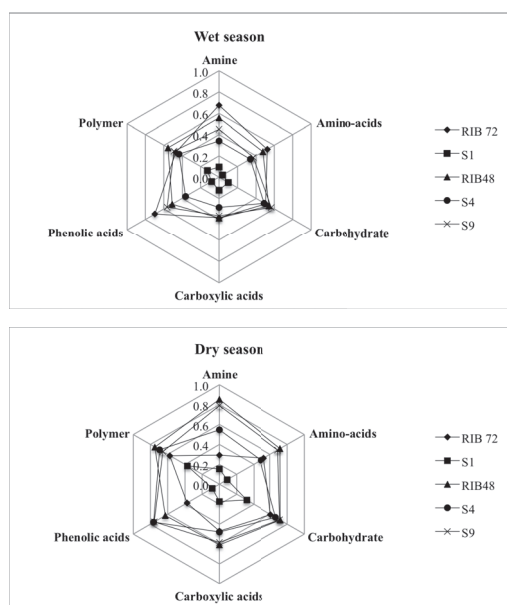


Fig 5.2: Potential metabolic capacity in different sites along the stream in the wet and dry seasons (as measured by Biolog® Ecoplate). The carbon substrates were grouped into 6 substrate families. The position on each of the six axes indicates the highest potential degradation rate of that group of substrates by the community after a 72-h incubation.

Bacterial community structure and diversity

Similar to metabolic capacities, the patterns of community structure were more related to local site specificities than to hydrological processes. Moreover, bacterial communities in both FL and PA fractions showed clear seasonal patterns in structure (Fig. 5.3A,B; AMOVA, $P < 0.01$). In the wet season, both FL and PA bacterial communities sampled at the sites harboring the highest DOC concentrations (i.e. RIB48 and S9) were highly similar, and those sampled at the two gauging stations (S1 and S4) showed alike structures. As observed above for the metabolic capacities, the communities sampled at the uppermost site (RIB72) did not follow this pattern. The FL fraction and PA fraction in the dry season had low similarity between sites along stream path from upstream to downstream in the study.

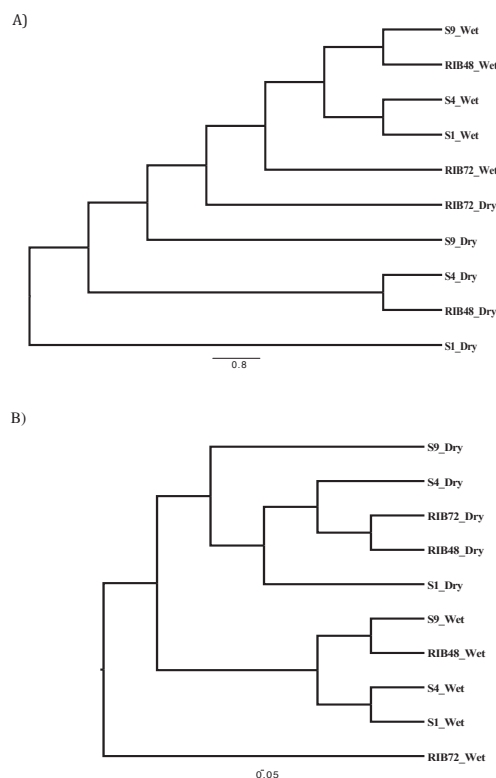


Fig 5.3: Bray-Curtis dissimilarity of community structure based on relative species abundances was used to cluster all samples in the dry and wet seasons for A) Particle-attached (PA) and B) Free-living (FL) fraction.

The 90,858 and 115,573 OTUs from the FL and PA fraction belonged to 35 and 39 different phyla, respectively (Fig S.5.3). The dominant bacterial phyla were affiliated to *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Firmicutes*. Overall, the relative abundance of OTUs belonging to *Firmicutes* was higher in the dry season than in the wet season for both FL and PA fraction.

In the PA fraction (Fig S.5.3A), OTUs belonging to *Proteobacteria* were relatively more abundant in the wet season than in the dry season. In the wet season, *Proteobacteria* dominated in all sites, in particular at the first gauging station S1 (85.4% of all OTUs were classified as *Proteobacteria*). OTUs classified as *Actinobacteria* and *Bacteroidetes* were also highly abundant phyla in the communities sampled in the wet season. Similar to the pattern found in the wet season, *Proteobacteria* found in the dry season dominated the communities at the outlet of the village (S9). Notably, the community structure from the dry season sampled at the second gauging station (S4) differed from the wet season, with a dominance of OTUs belonging to *Bacteroidetes* and *Firmicutes*, although they both were under the influence of teak plantation.

In the FL fraction (Fig S.5.3B), the relative abundance of OTUs belonging to *Proteobacteria* increased in the wet season, but decreased in the dry season from the uppermost station (RIB72) to the outlet of the village (S9). For this fraction, a large proportion (27.3% – 56% of total) of the OTUs found in the samples from the wet season could not be classified, except for the outlet of the village (S9) where *Proteobacteria* dominated. Similar to the metabolic capacity, the community structure sampled at the uppermost site (RIB72) in the wet season differed from the other communities due to a large extent to the relative dominance of OTUs belonging to *Bacteroidetes*. In contrast at the dry season, *Proteobacteria* dominated the communities sampled in all sites, followed with *Firmicutes* and *Bacteroidetes*. Remarkably, high relative abundance of OTUs belonging to *Firmicutes* (40%) was found at the outlet of the village (S9).

The richness and diversity of bacteria also did not increase from upstream to downstream and showed differences between sites (Table S.5.4). The richness of PA bacteria was highest at RIB48 and S9 as compared to the other sites in the wet season, whereas in the dry season it was highest at S1. For FL bacteria, the highest richness occurred at S9 in the wet season and at S1 in the dry season. The diversity of PA bacteria was high at RIB48 and S4 for both the wet and dry seasons. Similarly, the diversity of FL bacteria was high at S4 for both seasons (Table S.5.4).

DOC concentration was significantly, positively correlated with the richness and the diversity of PA community in the wet season (Correlation coefficient $R = 0.9$ and 0.81 , $P < 0.01$, respectively), whereas Sr was negatively correlated with Shannon diversity (Correlation coefficient $R = -0.8$, $P < 0.01$).

Impact of LU, the legacy of LU change and environmental variables on aquatic bacterial communities.

The first PLS regression analysis (data not shown) indicated that only the first axis was interpretable (axis 1, $Q_2 = 0.03$, axis 2, $Q_2 = -0.07$). The second PLS regression analysis indicated that the sample sites were grouped on the first axis according to site characteristics rather than according to seasonal variations (axis 1, $Q_2 = 0.19$, axis 2, $Q_2 = -0.002$). Fig 5.4 splits the graph of the explanatory variables into three groups (physicochemical measurements, LU change and its legacy) and that of the target variables (substrate activity, diversity indexes for the PA and the FL fraction). The positive (right) side of the correlation circle is associated with teak plantation (Teak 50 for LU change), LU change legacy of teak to crops (CropToTeak 50), from crops to teak (TeakToCrop 100), forest to crops (ForestToCrop 150), DOC, Sand Leptosol 150 and Sr (molecular weight). The negative (left) side of the circle is associated with banana plantation (Banana 50), fallow (Fallow 50 or CropOrFallow 50), low-drained luvisol (Luvisol 150) and swamp (Swamp 250 for LU and LU change) and $SUVA_{254}$ (DOM aromaticity).

The PLS analysis confirmed that metabolic capacities were high in the samples with high DOC inputs (RIB48, S9), and that PA bacterial diversity was strongly associated with the soil occupation

(mostly teak plantation) (Teak 50) and recent LU change from crop to teak plantations (CropToTeak 50) and from teak to crop (TeakToCrop 100) during 4 years (2012-2015). Though being sampled at the upland bog, the community structure in RIB48 was much more affected by surrounding LU (teak plantation) rather than swamp factors. The swamp area (Swamp 250) had high value of $SUVA_{254}$. On the other hand, the FL bacterial diversity was associated with the contemporary LU with banana plantation and fallow being important around the first gauging station (S1).

5.1. Vicinal land use change strongly drives stream bacterial community in a tropical montane catchment

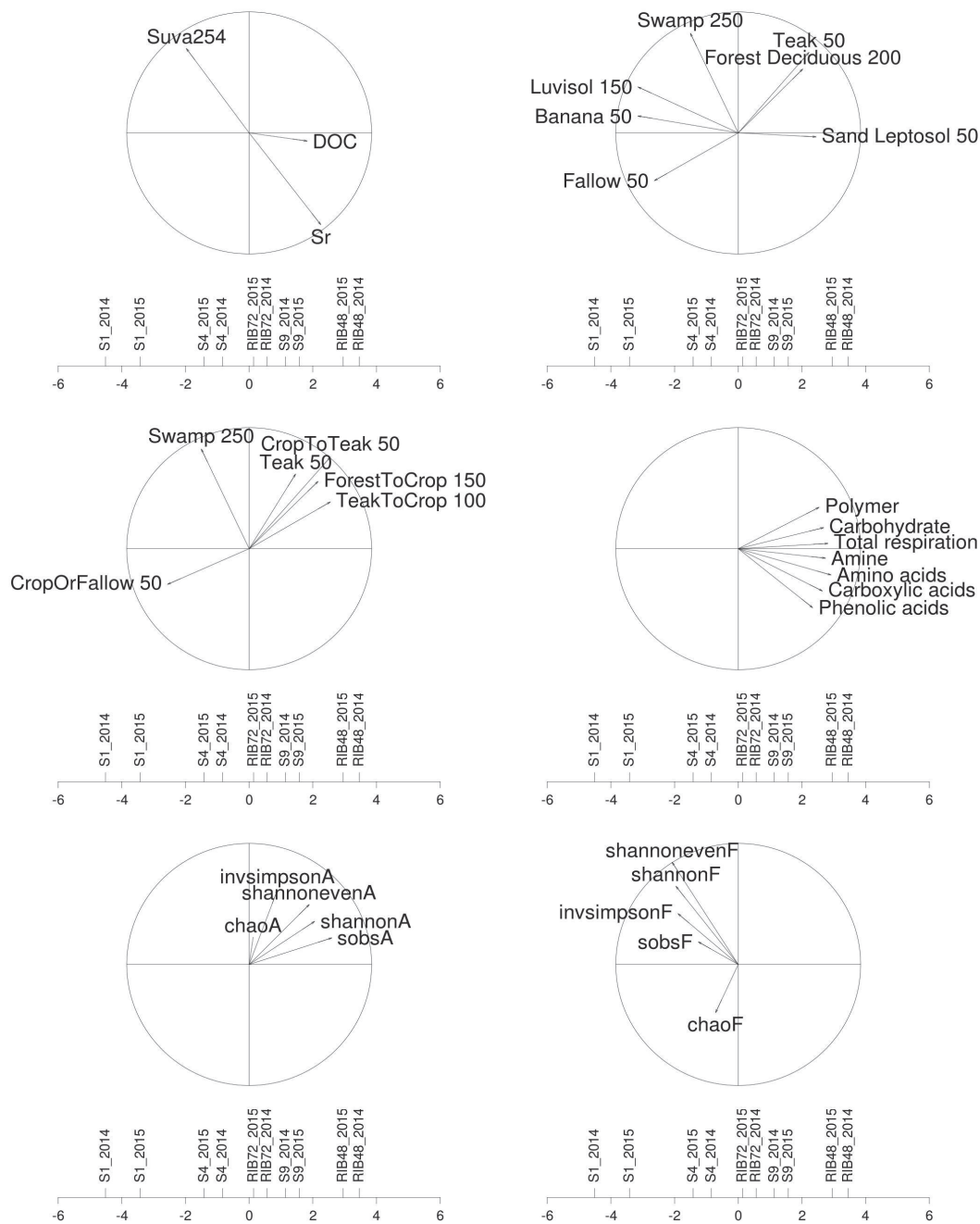


Fig 5.4: Correlation circles of partial least squares (PLS) regression, dividing explanatory variables into three groups in different graphs: physicochemical measurements (DOC, SUVA₂₅₄, Sr); soil type, land use change & its legacy; and the target variables including groups of carbon substrates; diversity indexes (Chao, Invsimpson, Shannon, Shannoneven and sobs: number of observed OTU) for Particle-attached (A) and Free-living (F) fraction. Only the first axis was interpretable (axis 1, $Q_2 = 0.19$, axis 2, $Q_2 = -0.002$). Sample sites from different seasons were grouped according to the first axis.

Co-occurring OTUs and stream environmental analyses

The OTU co-occurrence patterns were assessed in relation with DOC/CDOM characteristics using co-occurrence network analysis (Fig 5.5). C-scores and the properties of co-occurrence network are shown in Table 5.4. C-scores observed for both PA and FL fraction and both seasons, except for FL in the wet season, were higher than the C-score produced by the null model (Table 5.4). This indicated that pairs of OTUs tend to co-occur less often than expected by chance (*i.e.* there was segregation of OTUs) in the PA community in both seasons and in FL community in the dry season.

Table 5.4: C-score and typology properties of co-occurrence network for particle-attached (PA) and free-living (FL) fraction in the wet and dry season

	Dry - PA	Wet - PA	Dry - FL	Wet - FL
Observed C-score	1.29	0.62	1.35	1.5
Simulated C-score	1.28	0.59	1.31	1.49
<i>P</i> value	0.01	0.001	0.001	0.14
Number of nodes	170	281	182	114
Number of edges	1490	3853	1888	509
Connectivity	0.103	0.098	0.114	0.079

The number of nodes and edges of the co-occurrence network of PA was higher in the community sampled in the wet season, with 281 nodes (*i.e.* OTUs) and 3853 edges (*i.e.* significant positive or negative correlations), than in the dry season (170 nodes and 1490 edges; Table 5.4). The co-occurrence network of the FL community showed an opposite trend, with the community sampled in the dry season having a higher number of nodes and edges (182 and 1888, respectively) than that sampled in the wet season (114 nodes and 509 edges; Table 5.4). The connectivity between OTUs of co-occurrence network was higher during the dry season than the wet season for both PA and FL community (Table 5.4).

In general, the network of PA in the dry season was more separated and structured than the network of the wet season (Fig 5.5). OTUs (*i.e.* >0.1% relative abundance) that were found in at least two sites accounted for a large proportion (37.01%) in the network of PA in the wet season and only 15.88% in the PA network in the dry season (Fig 5.5). Moreover, there was more negative correlation between OTUs in the wet season PA network (37.3%) compared to the dry season (24.03%). OTUs that appeared in at least two sites were also more common in the network built from the FL community sampled during the wet season (34.21%) than in that the dry season (33.52%, Fig 5.5).

DOC/CDOM properties, LU occupation and the legacy of LU change were also included in the networks to decipher which abundant OTUs (*i.e.* >0.1% relative abundance) were related to these driving

factors. We found that DOC concentration was highly correlated with 59 OTUs including 16 OTUs in the dry and 43 OTUs in the wet season for PA network. These OTUs were mainly classified as *Alphaproteobacteria*, *Actinobacteria* and *Sphingobacteria*, and could be considered as well adapted to the two sites harboring the highest DOC concentration (*i.e.* S9 and RIB48). The fulvic acid and humic-like fluorescence components (C1 and C3) showed positive correlations with 24 PA OTUs that appeared in the second gauging station S4 and at the outlet of the village S9 in the wet season. Meanwhile, these components were positively correlated with 22 OTUs from the PA network in the dry season that occurred preferentially at the outlet of the village (S9) and that were in most cases classified as *Beta - Proteobacteria*. Moreover, DOC concentration and the tryptophan-like fluorescence component (C2) were negatively correlated with 9 OTUs that occurred at the first gauging station (S1) in the dry season (Fig 5.5A).

Compared to PA OTUs, fewer FL OTUs correlated with DOC: only 5 FL OTUs and 6 FL OTUs for the wet and dry season, respectively. These OTUs occurred preferentially in the upland bog (RIB48), the second gauging station (S4), and at the outlet of the village (S9) in wet season but only at S9 in the dry season. They were classified as *Alpha* or *Gamma - Proteobacteria* for wet season and *Bacilli*, *Clostridia* and *Alpha* and *Gamma - Proteobacteria* in the dry season. The fulvic acid and humic-like fluorescence components (C1 and C3) correlated with 16 OTUs that appeared mainly at the gauging station S4 and at the S9 outlet of the village in the FL community network for the wet season. These OTUs were classified as *Alpha*, *Gamma - Proteobacteria* and *Actinobacteria*. Similarly, in the dry season, fulvic acid and humic-like fluorescence components (C1 and C3) were associated with 10 OTUs at S9 that are classified as *Bacilli*, *Alpha*, *Gamma - Proteobacteria* (Fig 5.5B).

LU change legacy was correlated with various OTUs in the networks. Transition from teak plantation to crops in the past 4 years prior to sampling (TeakToCrop 100) appeared to be the most important as it was positively correlated with 17 and 31 PA OTUs for the dry and wet season at the outlet of the village (S9) and negatively correlated with 19 PA OTUs for both seasons. These OTUs were mainly classified as *Alpha*, *Beta*, *Gamma - Proteobacteria*, *Actinobacteria*, *Sphingobacteria* and *Flavobacteria*. The teak plantation (Teak 50) was positively correlated with 7 PA OTUs in the wet and 8 PA OTUs in the dry season (*Gamma*, *Beta - Proteobacteria*, *Sphingobacteria* and *Bacilli*) that were highly abundant in the upland bog RIB48. Banana plantation (Banana 50) was positively associated with 3 PA OTUs (2 *Gamma* and 2 *Beta - Proteobacteria*) occurring in the first gauging station (S1) at the wet season. Fallow (Fallow 50) showed also a positive relationship with abundant OTUs occurring in S1 at the dry season. They were mainly classified as *Deinococcales*, *Epsiloproteobacteria*, *Betaproteobacteria*, *Bacilli*, *Actinobacteria*, and *Sphingobacteria* (Fig 5.5A).

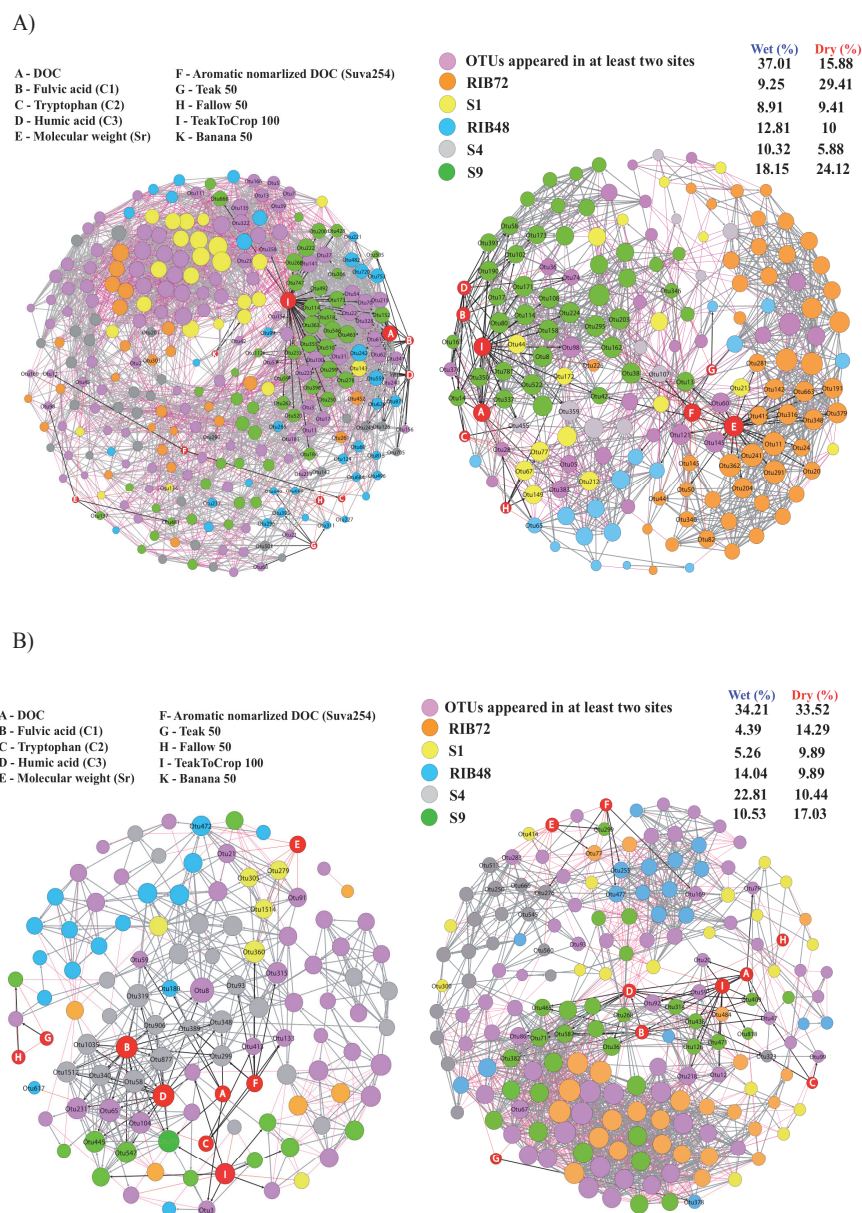


Fig 5.5: Co-occurrence networking of A) Particle-attached (PA) and B) Free-living (FL) fraction in dry (right side) and wet season (left side). A connection means that the correlation is strong (Spearman's $R > 0.8$) and significant ($P\text{-value} < 0.05$). Node color represents OTUs linked to a site where the relative abundance of OTUs was higher than 0.1% and was found for the site alone. OTUs that appeared in at least two sites with relative abundance higher than 0.1% were considered to build the network. The color of edges represents correlation between two nodes including negative correlation (red), positive correlation (grey). The positive correlation between OTUs and DOC/CDOM properties, LU change legacy was shown in black edges. Each node's size is proportional to the number of connections (degree).

5.1.4. Discussion

LU change as a driving factor of bacterial community structure

To assess which factors preferentially drove bacterial community diversity and structure, the legacy of LU, DOC/CDOM characteristics and seasonal variation were considered. Indeed, although only five sampling locations were sampled along the stream, they were chosen because they represent the main LU in this catchment, and because they were close to instrumented outlets. Although not fully satisfying from a statistical point-of-view, this sampling strategy was sufficient to cover the main representative LU in the catchment while at the same time avoiding redundancy. The results obtained from a small number of sites clearly indicate an influence of LU and its changes on the diversity of bacterial community. Moreover, the sampling strategy also allowed the comparison of the characteristics of DOC and stream bacterial community between the wet and dry seasons during an inter-storm period.

We hypothesized that the hydrological flow (and direction) more strongly influenced the structure of the bacterial community than vicinal LU change and its legacy. However, in contrast to our hypothesis, the results showed that although stream bacterial composition, structure and functioning varied with the seasonal variations of hydrological conditions, LU change and its legacy was the main determinant driving DOC/CDOM and the bacterial community along the stream. Many previous studies have indicated that seasonal hydrology is an important factor controlling freshwater bacterial communities (Crump et al., 2007; Nino-Garcia et al., 2016). Most studies have tended to focus on assessing the microbial assemblage in interconnected habitats or along hydrological fragmented habitats, and have provided conclusions as to the effect of local environmental factors (Fierer et al., 2007b) and regional processes (e.g. geographic distance and microbial dispersion) (Lindstrom et al., 2006; Fazi et al., 2013). The processes that affect bacterial structure in streams include regional hydrological connectivity, local environmental conditions and non-directed local processes (Freimann et al., 2015). In other words, the regional hydrological connectivity related to the dispersal of bacterial community (mass effect) or to changes of solute characteristics along the flow path shifts the bacterial community composition in streams. Local environmental conditions are important for forming the structure of the bacterial community when the mechanism of species sorting dominates over mass effect during the low flow. Therefore, we found here that the structure of the bacterial community was more related to local site specificities (local surrounding conditions) than to hydrological processes (flow path) because of low discharge, especially in dry season. However, few studies have considered these processes under effects of vicinal landscape factors on the local scale. The nutrient inputs from the surrounding agricultural watershed have been shown to increase DOC, influencing bacterial community structure (Song and Li, 2016). Bacterial community composition in the water column of a temperate river was driven by regional factors related to watershed LU (Hosen et al., 2017). Yet, the influence of LU is further complicated when cycles of change occur, such as when agricultural land converted to forest or when forest land is first converted to agriculture (Allan, 2004).

In the present study, the local morphological and physico-chemical characteristics were heterogeneous along the stream. For example, RIB48 was characterized by a gentle slope and muddy sediments in the upland bog whereas S4 was characterized by coarse sediments and a steep slope combined with a sinuous surface-subsurface water flow. The streambed sediments at a given spatial point along the stream-path principally originated from the surrounding hillslope soils that had their own vegetation cover, LU and LU legacies. Our results found that the upland bog station, RIB48, was associated with teak plots with high DOC and high richness and diversity of PA bacterial community. The environment of this station has undergone a crop / fallow transition to teak plantations during the four years prior to sampling (CropToTeak). Station S9 also had high DOC and was located in the village, but it was affected by a teak growing area subject to a transition towards crops during the four years prior to sampling (TeakToCrop). We also found high diversity of PA bacterial community at S4 that was surrounded with teak plantation although it was not significantly affected by teak plantation according to the PLS analysis.

Interestingly, LU change from crop/fallow to teak and from teak to crop/fallow led to increases in DOC and in the richness and diversity of the PA bacterial community. The crop/fallow transition to teak intensified drastically in this catchment in recent time. During the period 2008 to 2014, teak plantations covered up to 36% of catchment area, most of which were more than 3 years old and were characterized by limited understory vegetation cover and increasingly degraded soils (Ribolzi et al., 2017). According to this study, overland flow detached more soil particles in areas of teak plantation as compared to that generated under fallow. Moreover, the poor forest management may be the cause of the high rates of erosion rather than the nature of the teak itself (Fernández-Moya et al., 2014). In the Houay Pano catchment, most farmers frequently burn the vegetation understory in the teak plantations. Such land management results in increasing surface crusting, lower rain infiltration and higher overland flow and higher rates of soil erosion (Lacombe et al., 2017). During storm events, the overland flow from surrounding hillslope soils entered and accumulated in local streambed sediment in the HZ where exchange of surface and ground water stream occurs (Febria et al., 2012). This accumulation represents a potential source of DOC and soil PA bacteria from surrounding LU, which probably explains the observed high richness and diversity of PA bacteria at sites associated with the transition of crops to teak plantations, or teak plantation to crops. In other words, the presence of teak plantations, either currently or in the past, results in high DOC concentrations and richness and diversity of PA fraction in the stream. In addition, the high DOC concentrations and richness and diversity found at RIB48 and S9 are probably linked with the longer water residence times and riparian zones in the upland bog (RIB48) and from effluent from the village (S9). In contrast, the station S1 was adjacent to a banana plantation on low draining luvisol soils, which may be related to the low diversity of PA bacteria and low metabolic capacity at this site, especially in the wet season. Interestingly, the diversity of FL bacteria was high in this sample, as well as at the second gauging station (S4) which is surrounded by teak plantations. These

results suggested that PA bacteria community was more associated with LU change via local streamed sediments rather than FL bacterial community.

Seasonal difference in community structure and functioning of PA and FL bacteria

Seasonal changes inducing variability in water temperature, hydrological connectivity and organic matter quality and quantity are known to affect microbial diversity and composition in aquatic systems (Crump et al., 2003; Febria et al., 2012; Febria et al., 2015; Zeglin, 2015). However, these effects become more complicated in stream ecosystems due to their variable hydrology and associated potential for cell dispersal (Crump et al., 2012). For example, the seasonal shift in a temporary stream subject to hydrological fragmentation reduced microbial dispersion and affected the quality of DOM thereby shaping the bacterioplankton community composition (Fazi et al., 2013). Moreover, as transient storage does not vary with discharge (Ward, 2016), the proportion of bacteria that originate (or transit) the streambed will decrease at higher flow thus potentially leading to lower connectivity between stations along the stream.

We also observed differences in community structure between the dry and wet seasons for both PA and FL. There was a larger degree of separation in PA community structure between upstream and downstream in the dry season. As suggested above this indicates that the community structure of PA bacteria was more affected than FL bacteria by local environmental conditions. This has also been observed in a river-floodplain system where the PA bacterial community was more heterogeneous and more dependent upon changes in environmental conditions and terrestrial organic matter flow than was the FL bacterial community (Besemer et al., 2005). Our co-occurrence networks also showed that sampling sites along stream were more separated in the dry season than in the wet season. The OTUs of the PA network in the dry season showed site specificity, especially in RIB72 (the uppermost site) and S9 (outlet of the village). This can be explained by the longer residence time of local inputs of allochthonous carbon in the dry season leading to a greater spatial heterogeneity. A previous study has also suggested that freshwater inputs from the upper-river catchment and adjacent terrestrial runoff decreased the influence of localized factors on biological composition, although a clear spatial heterogeneity could be found during low inflow periods (Carney et al., 2015). In the wet season, more negative correlations appeared in the network (36.4%) indicating either competitive interactions or non-overlapping niches between microbes (Faust and Raes, 2012).

Relationship between DOC/CDOM and stream bacterial community under LU change

Our study emphasized that DOC concentration was an important factor that directly impacted stream bacterial diversity, richness and co-occurrence networks. Recent studies have shown that DOC is more correlated with the bacterial community in streams in the wet as opposed to the dry season (Febria et al., 2012) and that DOC plays a key role in the bacterial co-occurrence network of ponds and lakes

(Comte et al., 2016). Variations of DOM composition in soils and surface waters also influence bacterial community dynamics (Judd et al., 2006).

In our study, the role of DOC was stronger for the PA bacterial community than for the FL community. DOC was significantly correlated with diversity and richness of PA fraction (Correlation coefficient, $R > 0.8$, $p < 0.01$) while no correlation with the richness and diversity of FL fraction was found. Networking structure also suggested that DOC played an important role in connecting OTUs in the PA fraction network, especially in the wet season. In addition, DOC was correlated with fulvic acid and humic-like fluorescence components (C1 and C3). This suggests that DOC is of allochthonous origin, probably from upland soils. During precipitation events, overland flow exports the PA fraction and soil derived DOC from the soils surface into the stream thereby influencing stream DOC and bacterial community composition. A previous study also indicated that the soil runoff carried unique attached bacteria taxa associated with low molecular weight DOC into the HZ (Sabater et al., 1993).

The site at the outlet of the village (S9) that contained the most OTUs that correlated with high DOC and the components of fulvic acid and humic-like fluorescence components (C1 and C3) was impacted by the transition from teak to crops (TeakToCrop) in both the wet and dry networks. Potential bacterial metabolic capacity was also higher in RIB48 and S9. Although the humic-like component (C1) is considered to be recalcitrant DOC (Fellman et al., 2010; Khan et al., 2010), it may be more preferred as a carbon source by some bacteria than other more labile compounds (Rosenstock et al., 2005). Recalcitrant compounds which cannot be easily taken up by others are more reliable sources of carbon for these bacteria (Biasi et al., 2005). Meanwhile, the tryptophan-like component, considered as labile DOC for bacteria, was positively correlated with DOC aromaticity ($SUVA_{254}$) and high molecular weight (lower Sr). The high tryptophan-like fluorescence probably originates from dissolved amino acids in the non-living, high molecular mass, DOM pool released by the flushing of soil humic substances from the adjacent landscape in the stream (Yamashita and Tanoue, 2003; Fellman et al., 2009). Our results also showed that the tryptophan-like fluorescence component (C2) and $SUVA_{254}$ were negatively correlated with some PA - OTUs and positively correlated with FL - OTUs. This suggests that specific FL bacteria may have preferentially utilized this carbon substrate.

5.1.5. Conclusion and perspectives

This is the first study that gives a holistic view on the impact of LU change and its legacy on seasonal changes of stream DOC and PA and FL bacterial diversity and functioning in a tropical catchment under base flow. Although seasonal variation of DOC, bacterial community structure for both PA and FL fractions as well as bacterial metabolic capacity was found, LU change and its legacy in Houay Pano catchment is considered as driving factor for stream aquatic bacterial community. The PA fraction was much more affected by LU change and its legacy than the FL fraction in our study. LU therefore can exert both a direct and an indirect control on bacterial communities via streambed sediments and overland flow as a mechanism of erosion as well as a vector of solid particles from the

surrounding slopes. Moreover, it is interesting to note that changes in LU, particularly the transition between teak and crops that occurred between 2012 and 2015, and the presence of teak plantations led to an increase in the biodiversity of the PA fraction.

This study emphasized the importance of LU change and its legacy for a stream bacterial community in a tropical catchment. Indeed, proximal factors need to be taken account if we are to have a complete understanding of the drivers influencing stream bacterial community assemblages. This work also underlines the important role of LU management in upland tropical catchments, especially in developing countries where LU has drastically changed in recent years. Future work addressing the specific soil bacterial groups that characterize different LU and the local stream sediment community is essential if we are to fully understand the interactions between stream and soil communities in the catchment via local stream sediments in the hyporheic zone. It will also be interesting to conduct similar studies during stormflow periods as these hydrological events are known to have a high impact on sediment dynamics and on sediment bound bacterial transfer.

Supplementary Information

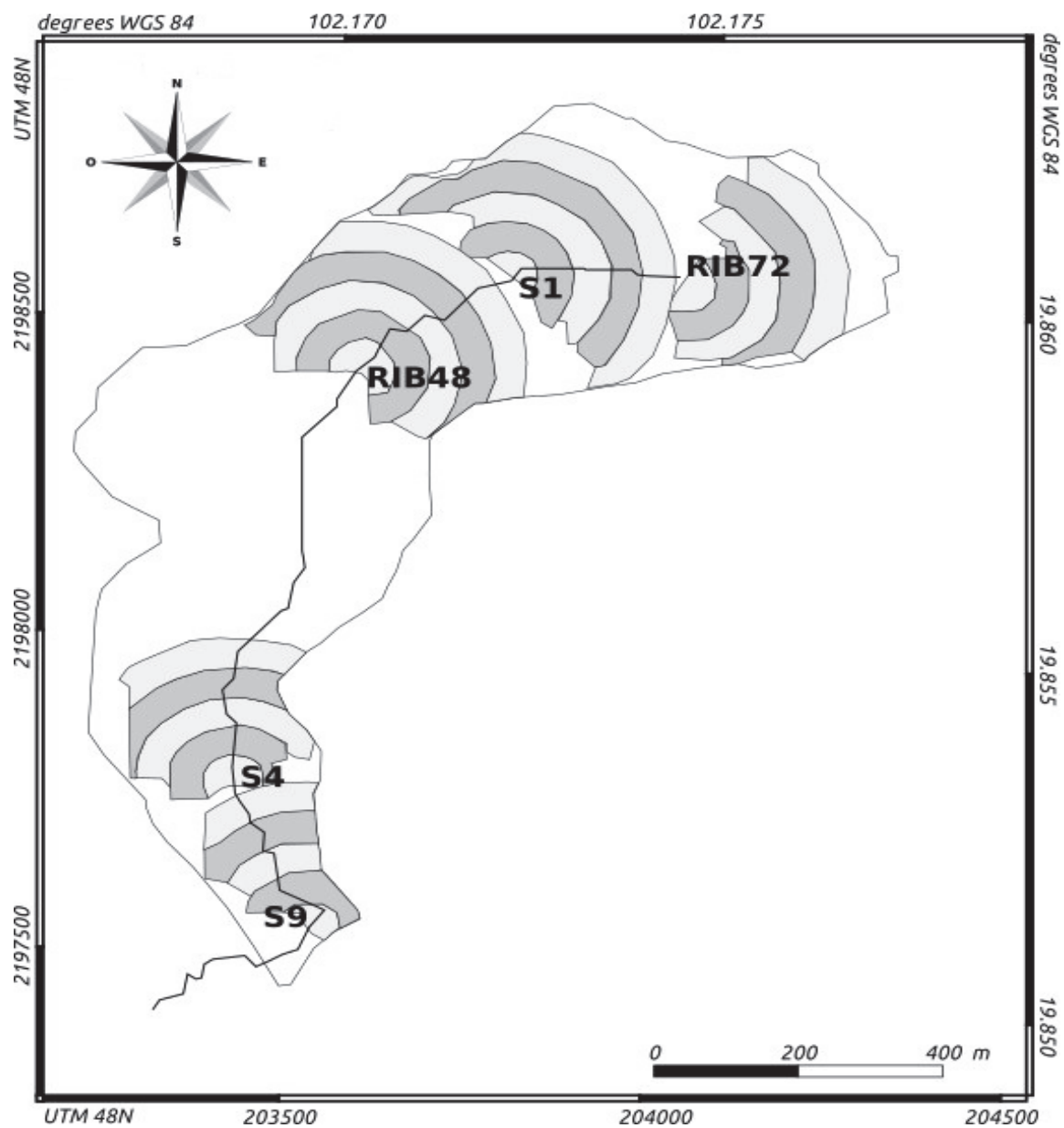


Fig S 5.1: Concentric drainage areas of sites

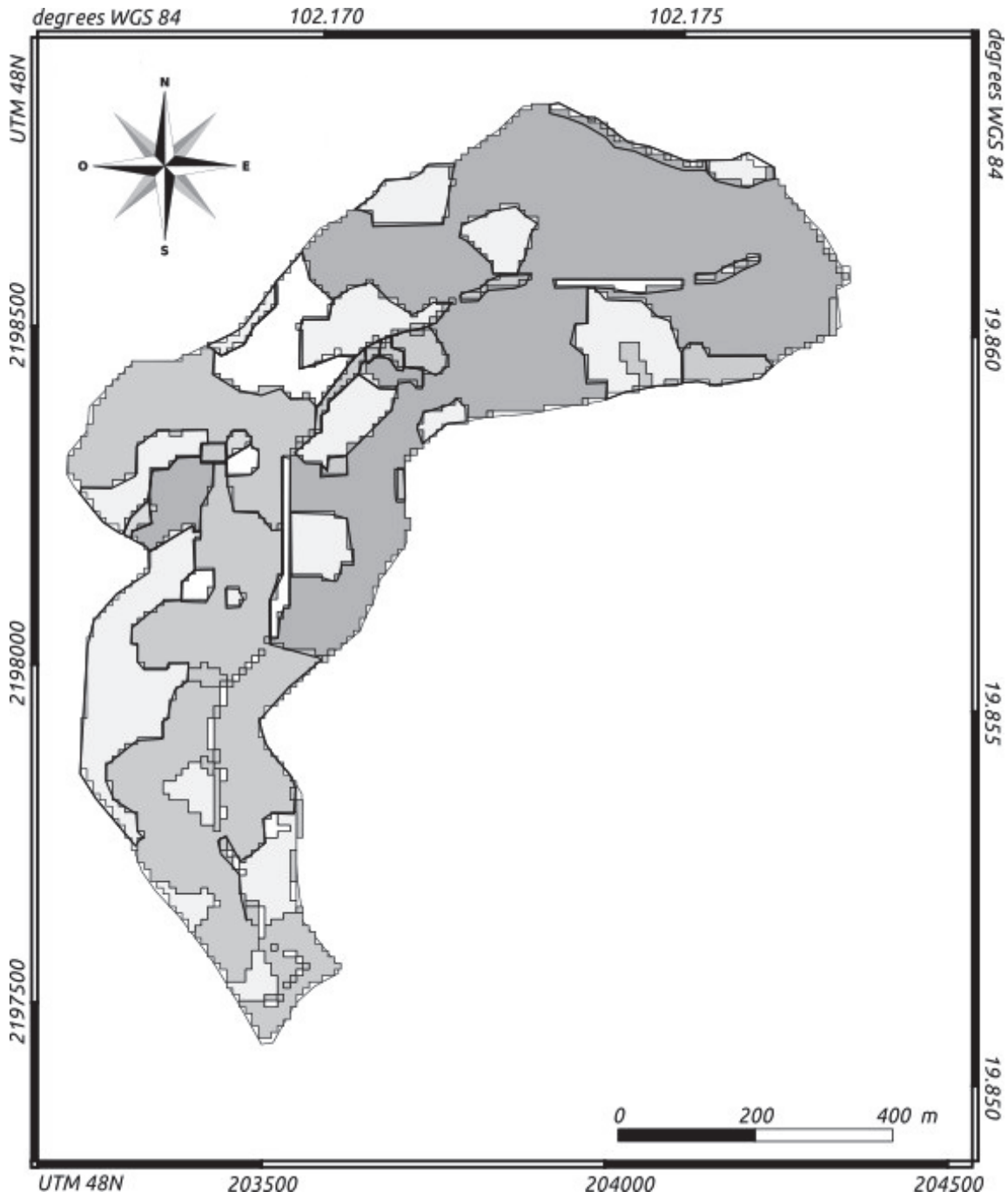
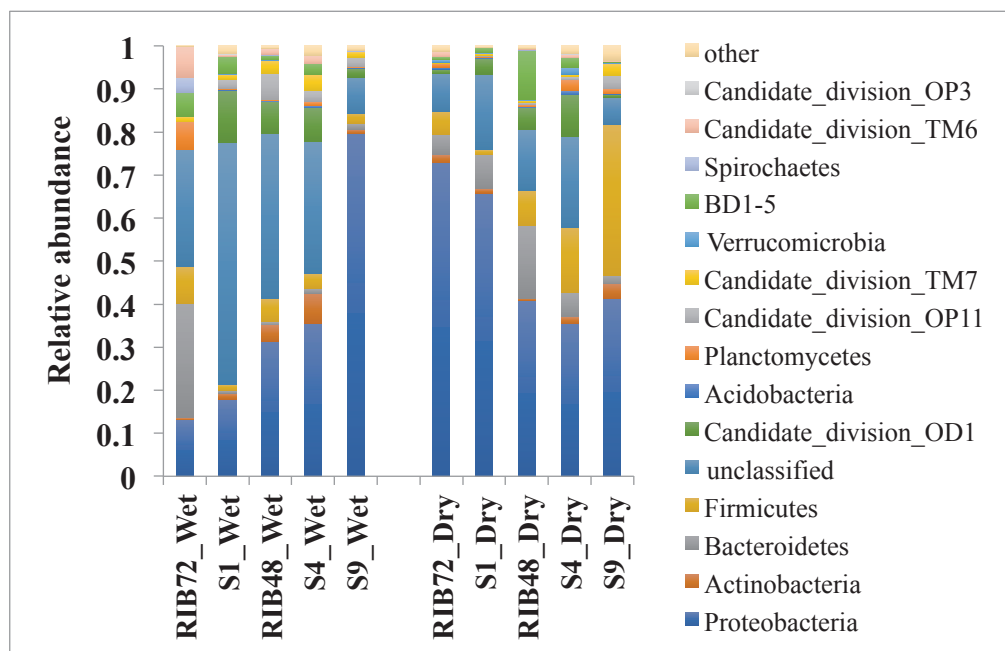
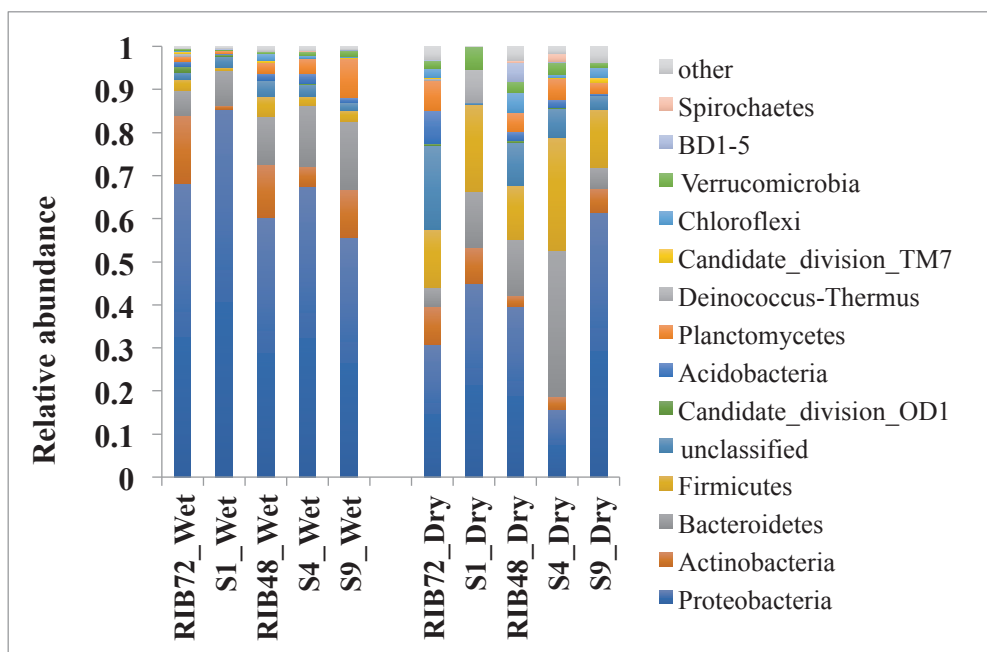


Fig S 5.2: Exploitation plots used for tracking changes in land use

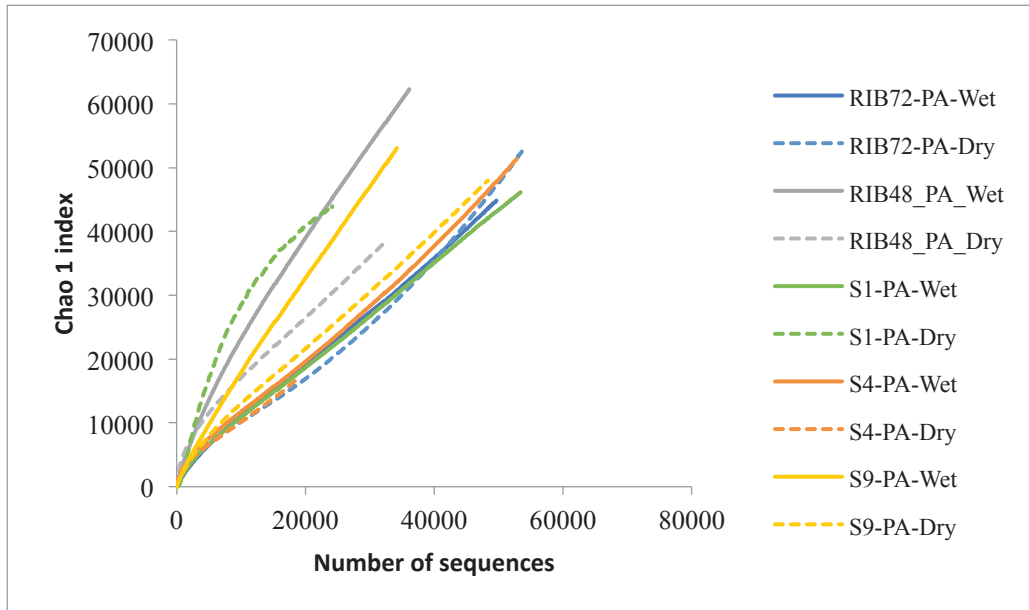
A) PA



B) FL

Fig S 5.3: Taxonomy of phylum level for A) PA and B) FL

A) PA



B) FL

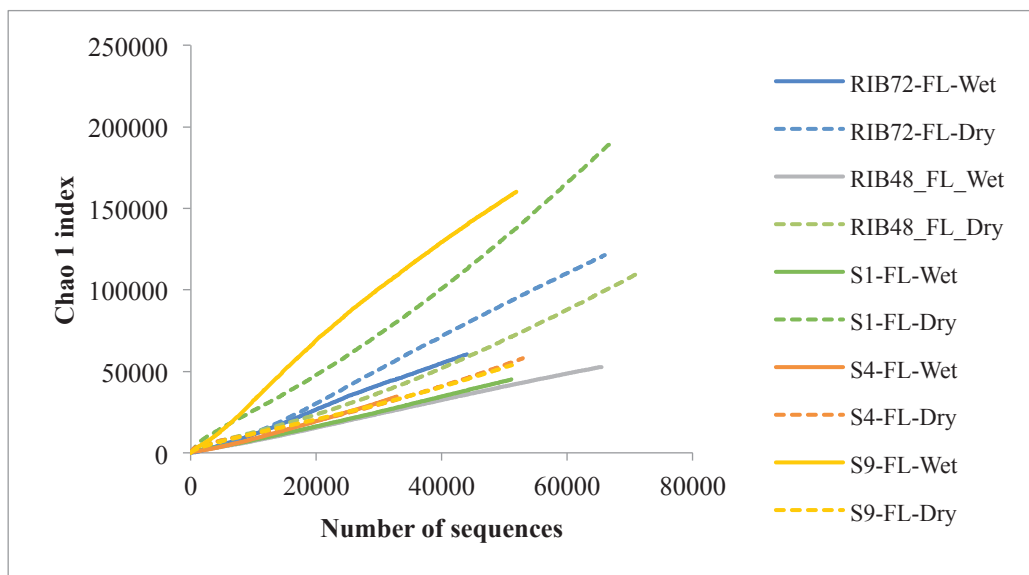


Fig S 5.4: Rarefaction curves estimating the effects of sampling on Chao 1 index for A) PA and B) FL

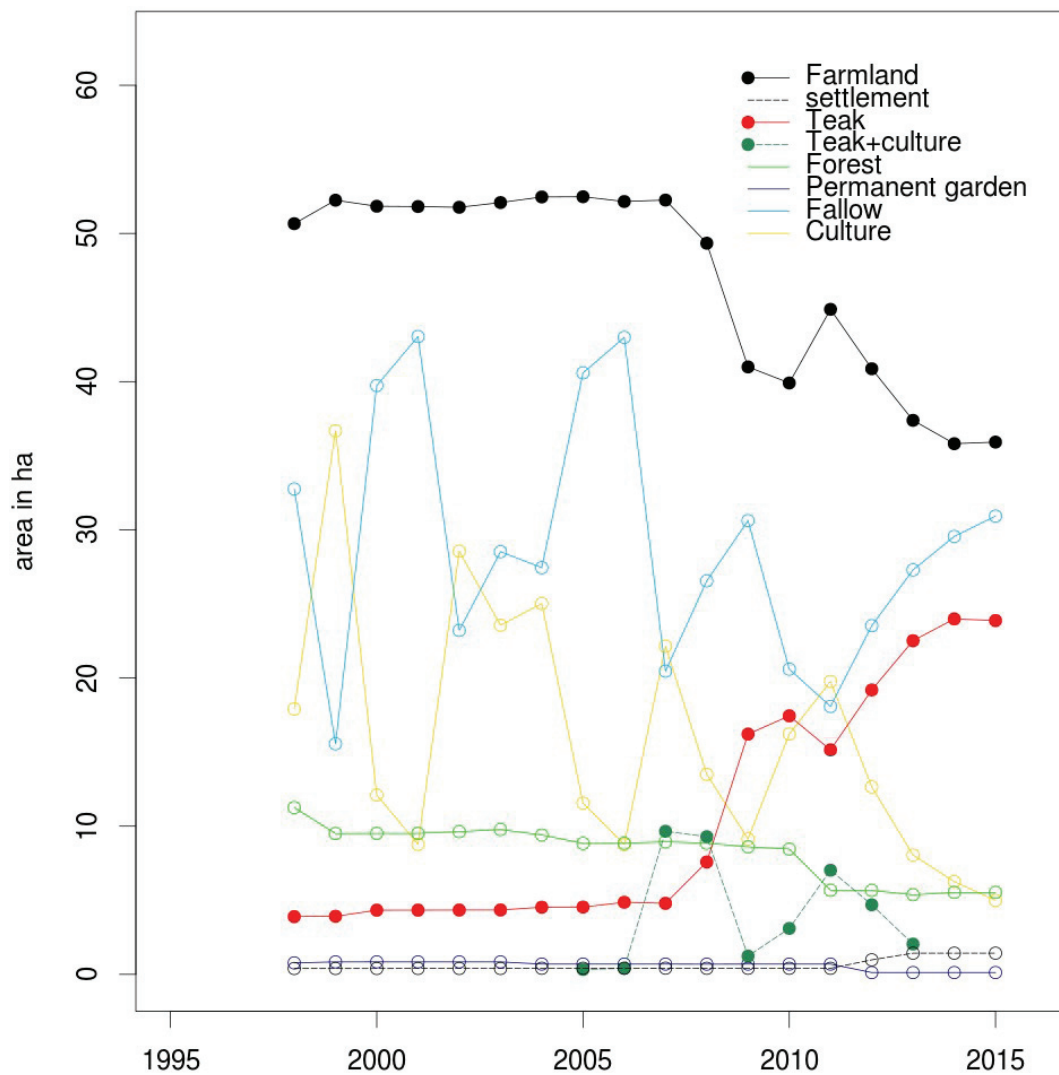


Fig S 5.5: Land use changes from 1998 to 2015

Table S 5.1: Correspondence between land use and simplified land use

Land use	Simplified land use
Banana	Culture
Broom grass field	Culture
Fallow	Fallow
Fish pond	Permanent garden
Forest (deciduous)	Forest
Forest (Dipterocarpe)	Forest
Job's tear	Culture
Maize	Culture
Sweet potato*	Culture
Teak	Teak
Temporarily unit	Culture
Upland rice	Culture
Vegetable garden and orchard	Culture
Village	Settlement
Swamp	Swamp

* LU absent in 2014

Table S 5.2: Land use transition during 4 years (2012-2015)

Type of transition	Surface in ha
Crop (or fallow)	33.7
Transition from crop to teak	3.0
Transition from teak to crop	1.5
Teak	19.5
Permanent garden	0.3
Village	1.3
Forest	5.2
Transition from forest to crop	0.5
Swamp	1.7

Table S 5.3: Soil categories

Type of soil	Surface in ha
Village	0.4
Luvisol	21.8
Alisol	32.3
Sand Leptosol	12.4

5.2. Impact of overland flow on particle-attached and free-living fractions during a flood event in an upland tropical catchment

Table S 5.4: Richness and diversity of the bacterial community in the wet and dry season

Season	Sample	Number of observed OTU (Sobs)	Chao	Invsimpson	Shannon	Shannoneven
Wet season	RIB72-PA	3815	17994.62	23.04	5.43	0.66
	S1-PA	3649	17408.75	59.40	5.53	0.67
	RIB48-PA	7062	36615.38	233.62	7.35	0.83
	S4-PA	5261	18326.16	124.34	6.77	0.79
	S9-PA	5127	30345.14	82.50	6.38	0.75
	RIB72-FL	1672	24279.78	35.26	4.41	0.59
	S1-FL	3682	14810.81	90.64	6.66	0.81
	RIB48-FL	3427	14007.80	27.46	6.11	0.75
	S4-FL	2847	17711.26	90.98	6.24	0.78
	S9-FL	5892	63446.70	15.69	5.82	0.67
Dry season	RIB72-PA	5841	15744.47	237.32	7.44	0.86
	S1-PA	1665	39889.29	24.00	3.87	0.52
	RIB48-PA	7907	24928.38	295.80	7.90	0.88
	S4-PA	5774	16578.24	417.90	7.60	0.88
	S9-PA	4821	20291.84	47.15	6.18	0.73
	S10-PA	2675	13292.24	4.14	4.03	0.51
	RIB72-FL	3935	26935.56	22.31	5.50	0.66
	S1-FL	7243	44041.05	51.37	6.81	0.77
	RIB48-FL	5091	21596.86	40.36	6.16	0.72
	S4-FL	6474	18338.58	243.44	7.69	0.88
	S9-FL	4776	19068.87	42.54	6.20	0.73
S10-FL	5979	18355.22	353.65	7.59	0.87	

In the section 5.1, I showed how stream DOC concentration and optical characteristics and bacterial community structure and metabolic capacity were impacted by hydrological processes at base and flow. Although we expected the streams to function as a “funnel” of particles from the different lands in the catchment, the vicinal LU change and its legacy were counter intuitively the main determinants driving stream bacterial community locally. LU caused both a direct and an indirect effect on bacterial communities via streambed sediments at base flow and overland flow at high flow as a mechanism of erosion as well as a vector of solid particles from the surrounding slopes.

In the next section 5.2, I propose to investigate how the terrestrial runoff from upland field may influence stream bacterial community for both particles attached (PA) and free-living (FL) fractions during a flood event.

5.2. Impact of overland flow on particle-attached and free-living fractions during a flood event in an upland tropical catchment

This chapter is in preparation for submission to the journal “Aquatic Science” as:

Huong T. Le, Thomas Pommier, Sylvain Huon, Olivier Ribolzi, and Emma Rochelle-Newall

Abstract

The export of allochthonous nutrient and bacteria originating from soil into stream water via overland flow can shift structure and function of stream ecosystem. Investigating how overland flow impacts dissolved organic carbon (DOC) concentration and stream bacteria community structure during a flood event is important to both soil and stream ecosystem health, but it is generally understudied, especially in tropical catchments. Here we measured DOC concentration and its optical characteristic (TSS), stream bacterial diversity and composition in the upstream, downstream and overland flow during a flood event. We found TSS and DOC concentrations increased following the peak of discharge. The decrease of the ratio of allochthonous and autochthonous DOC and higher humification indicated that soil originating DOC exported to stream during the flood recession. The ratio of the number of shared OTU at overland flow with total number of stream OTUs increased as the flood progressed in the upstream from 0.17 to 0.25 for the particle-attached (PA) fraction and from 0.01 to 0.04 for the free-living (FL) one. Moreover, the community structure of PA fraction at upstream and downstream was more similar to overland flow than was the FL fraction, suggesting that stream bacterial community was driven by dispersal of PA bacteria originating from soil during the flood recession. We also found that TSS, allochthonous DOC was the significant factors driving the composition of bacterial community, especially PA fractions during the flood event. In conclusion, the results emphasized that the effect of overland flow during the flood event on structure of stream bacterial community, especially PA, that may be in turn affect soil bacterial community and its functions for a long term. Considering the effects of the flash flood event play an important role in soil and stream management in a tropical catchment.

Keywords: flood event, overland flow, allochthonous DOC, stream bacterial community

5.2.1. Introduction

A flash flood in a stream is generated by a combination of high rainfall rate combined with rapid and efficient runoff production. Flash floods take place rapidly within a few hours of a rainfall event and have relative high peak discharge characterized by the dynamic of rainfall variability, and its complex interaction with basin characteristics (Syed et al., 2003). Individual flood can have an important role in many hydrologic processes such as sediment deposition along channels and in floodplain (Croke et al., 2013) and on the surface-groundwater interaction (Dahm et al., 2003; Nowinski et al., 2012). The amount and timing of overland flow occurring during a flood are regulated by basin shape and size (Syed et al., 2003), soil moisture (Ali and Roy, 2010), and surface roughness, crusting and vegetation (Valentin and Bresson, 1992; Poepl et al., 2012; Trevisani and Cavalli, 2016). Overland flow during floods redistributes material across the landscape mosaic when water, soil, nutrients and organic material are transferred across the landscape (Belnap et al., 2005). This mobilization of nutrients and carbon may increase during flooding due to interference with biochemical processes in the soil and the type of LU is recognized to affect the levels of nutrient loading to downstream ecosystems (Lamers et al., 2006; Banach et al., 2009).

Such biogeochemical change consequently impacts the stream biology, especially its microbial component that is prone to quickly respond to environmental variations. Stream microbial communities are shaped by different processes relating to local environmental factors or to dispersal mechanisms (Lindström and Langenheder, 2012). Dispersal mechanisms can become more important and outweigh the effect of local environmental factors (species sorting) from a mass effect perspective, especially at high flow (Langenheder et al., 2012; Adams et al., 2014). Bacterial community transported from soil into stream can easily adapt and persist in new habitats due to frequent and high dispersal rates (Adams et al., 2014). Dissolved organic carbon (DOC) from terrestrial sources, which can represent a significant input into rivers and stream during the high flow, influences microbial activity, growth and bacterial composition and diversity through selective local processes (Carvalho et al., 2003; Carney et al., 2015). The quantity and quality of allochthonous DOC drained from different soils and flushed into a stream play a critical role for aquatic bacterial growth (Kirchman et al., 2004; Agren et al., 2008). However, to our knowledge, the impact of overland flow, including the associated terrestrial DOC and bacterial community, on shifting stream bacterial diversity and structure during a tropical flood event remains unknown.

The Houay Pano catchment, is characteristic of tropical mountainous catchments in Southeast Asia in that it experiences heavy rainfall during summer monsoon, has steep slopes and is subject to rapidly changing LU with a move from shifting cultivation with annual crops towards teak plantations. In a recent monitoring of particle organic matter, total suspended sediment (TSS) and *Escherichia coli* numbers during a flood event in the wet season in this area, the authors emphasized the importance of surface sources with different LU (upland rice, fallow, teak plantation) in contributing the majority of

upstream suspended sediment (Gourdin et al. 2015, Ribolzi et al. 2015, Evrard et al. 2016). Importantly a small percentage of overland flow, *i.e.* one tenth of the total flood volume in the flood event, was responsible for more than two thirds of the downstream transfer of *E. coli* in this catchment. These results raised the question of whether overland flow from upland soil is the major factor controlling stream bacterial diversity and structure including particle attached (PA) and free living (FL) bacterial fractions during a flood event. The objective of this study was to understand how overland flow impacts DOC concentration, its optical characteristics and aquatic bacteria community structure during a flood event. So we collected stream water sampling at upstream (S4) and downstream (S10) stations at different times to assess the change of DOC and bacterial community during a flood event. Moreover, an additional sample of overland flow (S8) was collected to identify the bacterial community exported from overland flow to stream water during the flood course.

5.2.2. **Material and methods**

Study sites

The Houay Pano catchment is located 10 km south of Luang Prabang in northern Laos and covers an area of 60ha (Fig 5.6). It is a sub-basin of the Houay Xon system, which is a tributary of the Mekong River. The climate pattern is a wet-dry monsoon tropical climate with two particular seasons: the rainy season from mid-May to mid-October and the dry season from November to March. The average annual rainfall of $\sim 1300 \text{ mm year}^{-1}$ with 77% of the rainfall occurring during the rainy season. The watershed is characterized by the occurrence of about 30 storms during the wet season of which around 20 generates a flood in the stream. During these storms, rainfall reaches intensities of up to 100 mm h^{-1} over a time step of 6 min, similar to that commonly observed in other tropical systems (Ribolzi et al., 2015).

The main stem of the Houay Pano stream is equipped with gauging station S4, draining 60 ha (Fig 5.6). One additional station (S8) draining two sub-basins (0.6 ha each) that are connected to the main stream was also monitored. Houay Xon River (22.4-km^2 catchment), of which the Houay Pano stream is a tributary, is continuously monitored at station S10 just downstream of the confluence with the Houay Pano (Fig 5.6). The main LU in the 11.6-km^2 catchment drained by the S10 monitoring station are forests (covering 56 % of the total surface area), teak plantations (15 %) and cropland (23 %). In the upstream catchment drained by S4 (0.6 km^2), cropland (52 %) and teak plantations (36 %) dominate and are actively eroding (Evrard et al., 2016).

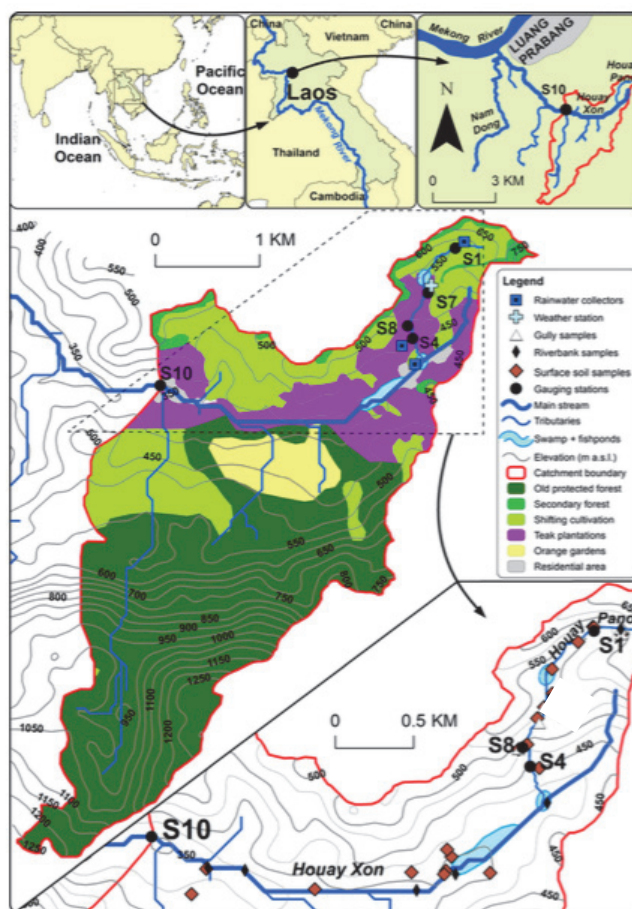


Fig 5.6: Location of study and sampling sites: in stream monitoring stations (S4, S10) and direct source sample locations and hillslope in situ sediment source sampling station (S8) (Evrard et al., 2016)

Hydrological measurement and water sampling

Water sampling took place during a flood event on June 16, 2014. Water samples were collected in plastic bottles by an automatic sampler (Automatic Pumping Type Sediment Sampler, ICRISAT) at stations S4, S10 (stream water) and S8 (overland flow). Ten samples ($n=1-10$) at S4, six samples ($n=2-7$) at S10 and two additional samples at S8 ($n=2, 8$) were collected at the different times over the course of flood.

Rainwater intensity was measured (with a 1-min time step) using an automatic rain gauge (Cimel). Stream discharge was measured at the catchment outlet using an automated recording station that consisted of a water level recorder (OTT, Thalimedes/SE200) and a V-notch weir. Electrical conductivity normalized to 25°C (EC) of each sample was measured with an YSI 556 probe (Ribolzi et al., 2015).

EC-based hydrograph separation

To separate overland flow and ground water flow during the flood event, a tracer-based approach was used with a simple mixing model with two reservoirs and the electrical conductivity (EC) of water as a tracer. It is described by the following equations (Ribolzi et al., 2015):

$$Q_{sw} = Q_{of} + Q_{gw}$$

$$Q_{sw}EC_{sw} = Q_{of}EC_{of} + Q_{gw}EC_{gw}$$

Where Q_{sw} represents the instantaneous stream water discharge at the catchment outlet, Q_{of} , the instantaneous discharge of overland flow and Q_{gw} , the instantaneous discharge of groundwater (expressed in $l\ s^{-1}$). EC_{sw} is the instantaneous EC measured in the stream. EC_{of} and EC_{gw} are the average values, respectively, for overland flow and groundwater during the storm event. EC_{of} was measured on samples of overland flow that were collected from the soil surface on hillslopes draining to the stream during the rainfall event (Patin et al., 2012). Since groundwater feeds the stream during inter-stormflow periods (Ribolzi et al., 1997), EC_{gw} was approximated from stream measurements carried out prior to storm event (S4.1).

Measurement of DOC, CDOM and TSS

A subsample of 200 mL of each sample was filtered through Whatman GF/F glass fiber filters to remove particles. For the determination of DOC concentration, duplicate 30 ml of this filtrate was kept in pre-combusted ($450^{\circ}C$, overnight) glass tubes, preserved with $36\ \mu L$ 85% phosphoric acid (H_3PO_4) and sealed with a Teflon lined cap. Samples were stored at ambient temperature and in the dark until measurement. DOC concentration was measured on a Shimadzu Total Organic Carbon (TOC) V_{CPH} analyzer following the method described in Rochelle-Newall et al. (2011). DOC concentration is expressed as mg of organic C per liter of water.

For CDOM, 100-ml filtered samples were stored in pre-cleaned 125-ml amber glass bottles sealed with Teflon lined caps. After collection, the samples were stored frozen ($-20^{\circ}C$) until measurement. Before the optical measurements, the samples were thawed slowly to room temperature and re-filtered at $0.2\ \mu m$ (Sartorius Minisart NML Syringe filters) to remove particles. CDOM absorption was measured with a spectrophotometer (Analytica.Jena Specord 205 UV-VIS) from 200-750 nm using a 10 cm quartz cell and Milli-Q water as the blank.

$SUVA_{254}$ (specific UV absorbance) provides a measure of aromaticity of DOM (Weishaar et al., 2003) and was computed by dividing the UV absorbance at 254nm (m^{-1}) by the concentration of DOC ($mg\ C\ L^{-1}$) (Hood et al., 2006). The spectral slope ratio, S_R was also calculated as the ratio of the slope of the shorter UV wavelength region (275–295 nm) to that of the longer UV wavelength region (350–400

nm) (Helms et al., 2008) and was obtained using linear regression on the log-transformed spectral ranges (Yamashita et al., 2010). Humification (HIX) was calculated from excitation 255 nm as the ratio of the peak area under each curve at emission 434-480 nm and 300-346 nm. HIX indicates the humic content of DOM and ranges from 0 to 1 (Ohno, 2002).

Fluorescence index (FI) was measured as ratio of fluorescence emission intensities at wavelength region of 470:520 nm excites at 370 nm. This value around 1.3 suggests a dominant terrestrial, higher-plant DOC sources and values around 1.8 suggest a predominant microbial DOC sources (McKnight et al., 2001; Cory and McKnight, 2005).

Excitation – Emissions – Matrices (EEM) measurements were made on a Gilden Fluorosens fluorometer using a 1 cm quartz cuvette with 5nm bandwidths for excitation and emission at an integration time of 100 ms. Excitation scans were made over a range of 200 to 450 nm at 5 nm increments and emission from 220 to 600 nm at 2.5 nm increment. EEMs were corrected for inner filter effects and the manufacturers' machine correction was applied. EEM fluorescence of Milli-Q water blank was subtracted from that of sample EEM, and EEM converted to RU units.

PARAFAC analysis was carried out in MATLAB (version R2016a 9.0.0) with the DOMFluor toolbox for MATLAB (Murphy et al., 2013) in order to decompose the fluorescence signal into a series of tri-linear structures. Processing data was done to minimize the impact of scatter lines that caused some mathematical difficulties, including removal of Rayleigh and Raman scatter. EEM wavelength ranges were reduced to excitation 290-450 nm and emission 300-600 nm. PARAFAC model generated 3 components using EEMs from our samples (n=19). The three-component model (C1-C3) was validated using split-half and random initialization methods.

Total suspended sediment (TSS) samples were grounded with an agate mortar, weighed and packed into tin containers (5x9 mm).

Assessment of bacterial diversity

DNA extraction and 16S rRNA gene sequencing

Fifty milliliters of each sample was first filtered through a 3µm pore size filter (Polycarbonate Whatman) and a 0.2µm (Polycarbonate Supor) filters to separate Particle-attached (PA) fraction (>3.0µm) from Free-living (FL) fraction (<3.0µm and >0.2µm) (Crump et al., 1999). All filters were stored at -20°C until DNA extraction that followed a modified protocol adapted from (Fuhrman et al., 1988). Briefly, the filters were cut in two halves using ethanol cleaned scissors and placed in separate Eppendorf® tubes. One tube was placed on ice and 525 µl of lysis buffer was added to start breaking Gram negative bacterial cells. The other tube was stored at -20°C as a back-up filter. Three cycles of freeze-thaw switches (65°C for 2 min – ice for 5 min) were then performed to facilitate cell membrane breakage. Then 0.5 g of glass beads (Ø=0.5 mm) was added into the tubes. The tubes were shaken for 45 s at 6 m s⁻¹ using a Fastprep® (Millipore, Fastprep®-24, USA) and were incubated at 4°C for 5 mins; these

5.2. Impact of overland flow on particle-attached and free-living fractions during a flood event in an upland tropical catchment

steps were performed twice. 11 μl of Lysozyme (1 mg mL^{-1} , final concentration) was added to the tube and left for 30 min at 37°C to break down bacterial cell walls. Sodium dodecyl sulfate (SDS 10%) and proteinase K (final concentration, 100 $\mu\text{g mL}^{-1}$) were added to the tubes and were incubated at 55°C for 2 hours under constant shaking conditions (180 rpm) to remove the lipid membrane. A sodium chloride (NaCl 5M) and a cetyltrimethylammonium bromide (CTAB) solution (final concentration, 1% in a 0.7 M NaCl solution) was added to the tubes, mixed and incubated at 65°C for 10 min to separate DNA from protein. Nucleic acids were then extracted twice from digestion products with phenol-chloroform-isoamyl alcohol (25:24:1); the aqueous phase containing nucleic acids was kept and purified by adding phenol-chloroform-isoamyl alcohol (25:24:1). After isopropanol (0.6 volume) addition, the nucleic acids were precipitated at -20°C for 12 h. After centrifugation, the DNA pellet was rinsed with 99% pure ethanol to remove the salt previously added. The samples were spun 20 min at maximum speed (15,000 rpm) at 4°C and the supernatants were removed. The DNA pellets were dried in a Speed Vac® (Labconco, USA) for 10 min and re-suspended in 50 μl of molecular cleaned distilled water. Nucleic acid extracts were stored at -20°C and sent overnight to Molecular Research Laboratories (Texas, USA) for further PCR amplification, product cleaning, library construction and high throughput sequencing.

The PCR primers 515F (5'-AGRGTTTGATCMTGGCTCAG-3') and (5'-806R GTNTTACNGCGGCKGCTG-3') (producing a 291bp amplicons) (Capone et al., 2011) with sample-specific barcodes on the forward primer were used to amplify the V4 variable region of the 16S rRNA gene. Thirty (30) cycles of PCR were performed using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) under the following conditions: 94°C for 3 mins, followed by 28 cycles of 94°C for 30 seconds, 53°C for 40 seconds and 72°C for 1 minute, after which a final elongation step at 72°C for 5 mins was performed. After amplification, PCR products were checked in 2% agarose gel to determine the success of amplification, the relative intensity of bands and to check the expected amplicon size (291bp). The samples included in this study were pooled together in equal proportions based on their molecular weight and DNA concentrations. Pooled PCR products were purified using calibrated Agencourt AMPure XP magnetic beads according to the manufacturer's guidelines. Briefly, the pooled PCR products were incubated with magnetic beads (1.8:1 ratio of beads to sample) that pull down the PCR product to the bottom of a tube for subsequent washing steps. The clean PCR product is then release from the magnetic beads for elution. The pooled and purified PCR product was then used to prepare a DNA library by following Illumina TruSeq DNA library preparation protocol. Sequencing was performed at MR DNA (www.mrdnlab.com, Shallowater, TX, USA) on an Illumina MiSeq machine following the manufacturer's guidelines.

Sequences processing and data analysis

The MOTHUR software (v. 1.33) (Schloss et al., 2009) was used to process 16S rRNA gene sequence reads following the SOP. Short reads (<250 bp) and reads with ambiguous primer or barcode

sequences were discarded. Corresponding reads were paired in single sequences with an average length of 486 bp. Sequencing errors were reduced by aligning remaining reads to the SILVA database (Pruesse et al., 2007), screening the alignment to the overlapping region, and pre-clustering sequences distant by < 2bp. Chimeric sequences were identified using the integrated version of UChime (Edgar et al., 2011) and removed accordingly. To avoid misinterpretation, sequences that were classified as "Chloroplast", "Mitochondria", or "unknown" lineages were removed before clustering into Operational Taxonomic Units (OTUs). All samples were subsampled to 10,000 sequences before clustering into OTUs with a pairwise distance < 0.03 substitutions per nucleotide with the average neighbor method and considered for further analyses. Taxonomic assignments were performed on the alignment of consensus sequences with the RDP database (Cole et al., 2005).

Alpha and Beta diversity

Rarefaction curves were calculated for all samples using the MOTHUR software (v.1.33). Nonmetric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarity were also calculated to estimate the dissimilarity in structure between all samples using the *metaMDS* command from the *vegan* package in R (Oksanen et al., 2017). A canonical correspondence analysis (CCA) was performed to determine the relationship between the significant environmental variables and stream PA and FL community structure. Only OTUs with the total number of sequences >10 were included in CCA.

The relationships between environmental factors (DOC concentration, its components, TSS) and bacterial OTUs were analyzed using network analysis for PA and FL separately. A correlation matrix was constructed by calculating all possible pairwise Spearman's rank correlations. Only OTUs with the total number of sequences > 10 and its occurrence frequency shown at least 5 samples were taken into consideration. All results with Pearson correlation coefficients (*i.e.* $R > 0.7$) and significance levels ($p < 0.05$) were used to build the network. Only positive correlation was shown in the network. The nodes in each network represent OTUs and environmental factors and the edges that connect these OTUs show the correlation coefficients between OTUs. The size of nodes related to the number of sequences of OTUs. Networks were visualized in Gephi v. 0.9.1 using the Fruchteman-Reingold layout algorithm (Bastian et al., 2009).

Statistical analyses

Correlations between environmental factors were estimated using the Pearson correlation coefficient with the *Hmisc* package in R (Harrell, 2018). ANOSIM test was performed with Bray-Curtis dissimilarity using *vegan* packages in R to determine the significance of the difference in bacterial community structure. The *env.fit* function was applied to the Bray-Curtis dissimilarity matrices (*vegan* package in R) (Oksanen et al., 2017) to evaluate the significance of relationship between PA and FL bacterial structure and the environment factors (DOC and CDOM). All differences were considered significant when the p-value of the tests was lower to 0.05.

5.2.3. Results

Description of discharge over the flood

At the upstream location (S4), the maximum of rainfall intensity occurred between 13:34 pm and 13:35 pm and reached 60 mm h⁻¹. The peak of flood occurred at 14:18 pm with discharge of 33.3 L s⁻¹ with 21.3% of overland flow. The peak of overland flow occurred from 14:33 to 14:35 pm with 8.8 L s⁻¹. At the downstream location (S10), rainfall peaked at 13:42 with an intensity of 156 mm h⁻¹. The peak of discharge and overland flow achieved at 14:00 pm (S10-5) (543.1 L s⁻¹) with 59.8 % of overland flow (Fig 5.7).

Environmental variables

The concentration of TSS at S4 increased from 17.5 mg L⁻¹ before flood to 1174.7 mg L⁻¹ at the peak of the flood event and then decreased to 191.9 mg L⁻¹ at the end of the flood (Fig 5.7). DOC concentration at S4 increased with flood discharge from 2.6 mg L⁻¹, and achieved the highest value at 6.2 mg L⁻¹ at 15:10 pm after the peak of flood then it decreased to 4.2 mg L⁻¹ toward the end of the flood. Similarly, FI, which indicates the ratio between allochthonous and autochthonous DOC, gradually decreased during the rising flood, indicating more allochthonous DOC was found in the water. FI thereafter increased as the overland flow decreased. Humification (HIX) also gradually rose from 0.18 to 0.54 mg L⁻¹ with the increase of allochthonous DOC, meanwhile the aromaticity of DOC (SUVA₂₅₄) had a decreasing trend at the time of high allochthonous DOC. Sr, the inverse of molecular weight of DOC, remained relatively stable from 0.91 to 1.02 during the flood event. Noticeably, the molecular weight of DOC was lower during the flood compared to base flow.

Downstream at S10, TSS reached a peak of 2195 mg L⁻¹ following the peak of discharge and then declined to 409.3 mg L⁻¹ at the end of flood event. Similarly, DOC concentrations and HIX achieved their peaks with 4.4 and 0.39 mg L⁻¹ respectively at the same time. More allochthonous DOC was also surveyed at this time due to low FI (1.12). SUVA₂₅₄ fluctuated from 4.97 to 6.18, but tended to increase with increasing DOC and discharge flow (Fig 5.7). Sr, the inverse of molecular weight, varied from 0.85 to 1.05, similar to the values at S4.

In general, DOC concentrations during flood event were higher than during base flow as measured prior to the flood event (Fig 5.7). During the progress of flood, DOC concentration, the aromaticity of DOC (SUVA₂₅₄) and humification (HIX) at the upstream site (S4) was higher than that at the downstream site (S10), in contrast to what was observed for TSS.

PARAFAC model was applied and three components were found to describe the characteristics of DOC. Component 1 (C1) had a peak at excitation/emission (Ex/Em) wavelength of 340/457 that correspond to the component of UVC humic-like (peak C) (Fellman et al., 2010). Component 2 showed a peak of 420/475 for Ex/Em wavelength that is similar to the humic acid fraction (Yamashita et al., 2011).

Component 3 had a Ex/Em peak of 300/352 that found as Tryptophan-like fluorescence (Coble, 1996; Yamashita and Tanoue, 2003).

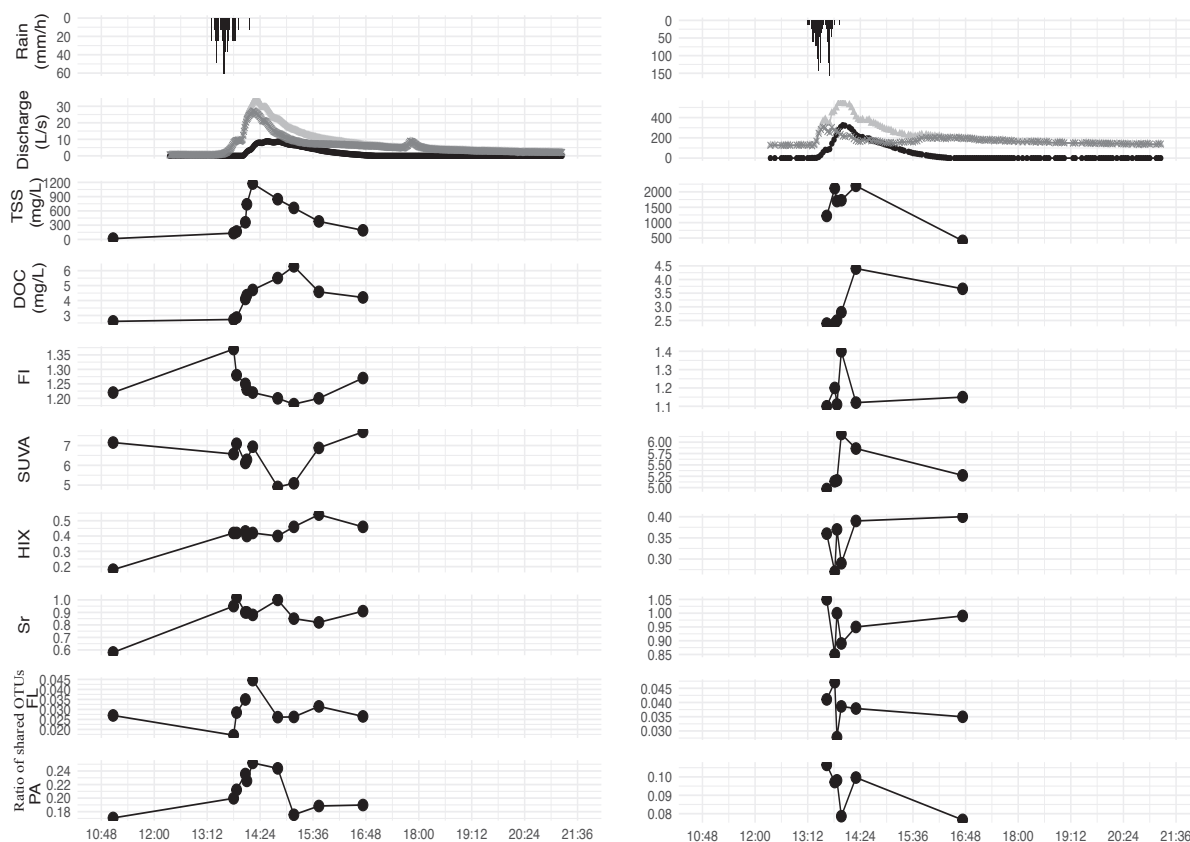


Fig 5.7: Environmental variables measured during the flood at the upstream S4 (left) and the downstream S10 (right) stations. From the top: rainfall intensity, stream discharge (Groundwater (GW) in grey line and overland flow (OLF) in black line), total suspended solid (TSS) concentration, Dissolved organic carbon (DOC), Fluorescence index (FI), the aromaticity of DOC (SUVA), Humification (HIX), the inverse of molecular weight (Sr), the ratio of shared OTU number with overland flow in the stream water for particle attached (PA) and for free-living (FL). Each dot indicates a sampling event during the continuous measurements of rain intensity and discharge for S4 (1-10 samples) and S10 (2-7 samples).

Bacterial community during the flood event

Diversity of PA and FL bacteria

The estimated Chao richness and Shannon diversity of the PA bacterial community was higher than the FL bacterial community in stream during the flood even (Fig S.5.6 and Table S.5.6). These values were higher during the flood event for both the PA and FL fractions compared to base flow (S4.1). However, the Chao richness and diversity of the PA and FL fraction fluctuated during the flood event (Fig S 5.6 and Table S 5.6).

Shared OTU between overland flow and stream water

The number of OTUs shared between overland flow (S8) and stream water (S4 and S10) and then the ratio of shared OTUs per total number of OTUs in stream over the flood was calculated at different times during the flood to indicate the contribution of bacterial community from overland flow to stream water during the flood event (Fig 5.7). At S4, this ratio increased with discharge flow and overland flow at the beginning of the flood recession, from 0.17 to 0.25 for PA and from 0.01 to 0.04 for FL. The values then decreased at the end of the flood. At S10, this ratio for the FL fraction varied slightly from 0.03 to 0.04 during the course of flood. The value for the PA fraction tended to decrease at the beginning of the flood event, thereafter it peaked again (0.09) together with the peaks of DOC, TSS and HIX. In addition, this ratio of shared OTU with overland flow in the stream water was higher in the PA than the FL fraction in both S4 and S10. This ratio for the PA fraction at upstream (S4) was higher than at downstream (S10).

Bacterial community structure

NMDS analysis for all samples indicated that there was a significant separation of community structure at the different sites and between the PA and FL fractions during the flood (ANOSIM, $R=0.5$, $P=0.001$). The PA fraction at S4 and S10 was more similar to overland flow (S8) than the FL fraction (Fig 5.8). The FL fraction was more separated between upstream (S4) and downstream (S10) than the PA fraction (Fig 5.8).

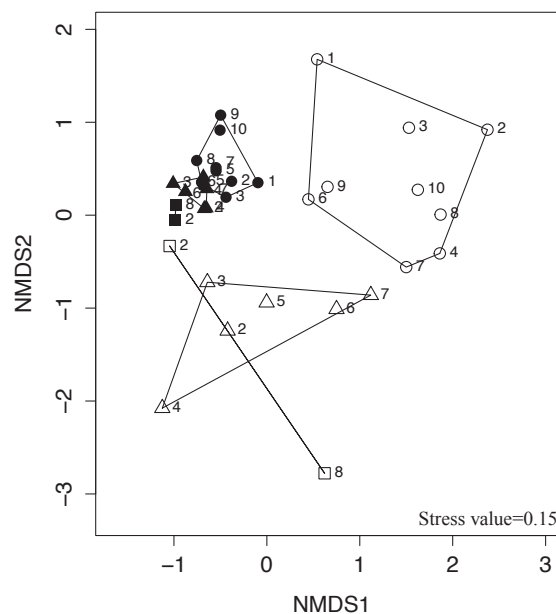


Fig 5.8. NMDS based on Bray-Curtis distance for all samples in different sites for both PA (filled symbols) and FL (open symbols) fractions during the flood. Circle: S4 (samples: 1-10), triangle: S10 (samples: 2-7), square: S8 (sample 2, 8). Note: due to low sequence cover, the sample S4-5 for FL was removed.

Effect of environmental factors on bacterial community structure

The relationships between environmental factors and the stream bacterial community were tested in canonical correlation analyses (CCA) and Monte Carlo permutation tests (999 permutations) for the stream bacterial community PA and FL fractions separately (Fig 5.9, Table S 5.7). The cumulative percentage of variance of relationship between the bacterial community and environmental factors indicated that the first and second canonical axis explained 27.6% and 21.8% of this variance, respectively, for the PA fraction, and 17.2% and 16% of this variance, respectively, for the FL fraction. DOC, the humic component (C1) and TSS were significantly correlated with the shift in spatial bacterial structure between upstream and downstream for both PA and FL fractions. However, the temporal shift of PA fraction in the upstream (S4) during the flood event that was driven by DOC and its components was clearer than the FL fraction because the PA community structure changed together with the increase of DOC as the flood rose (Fig 5.9).

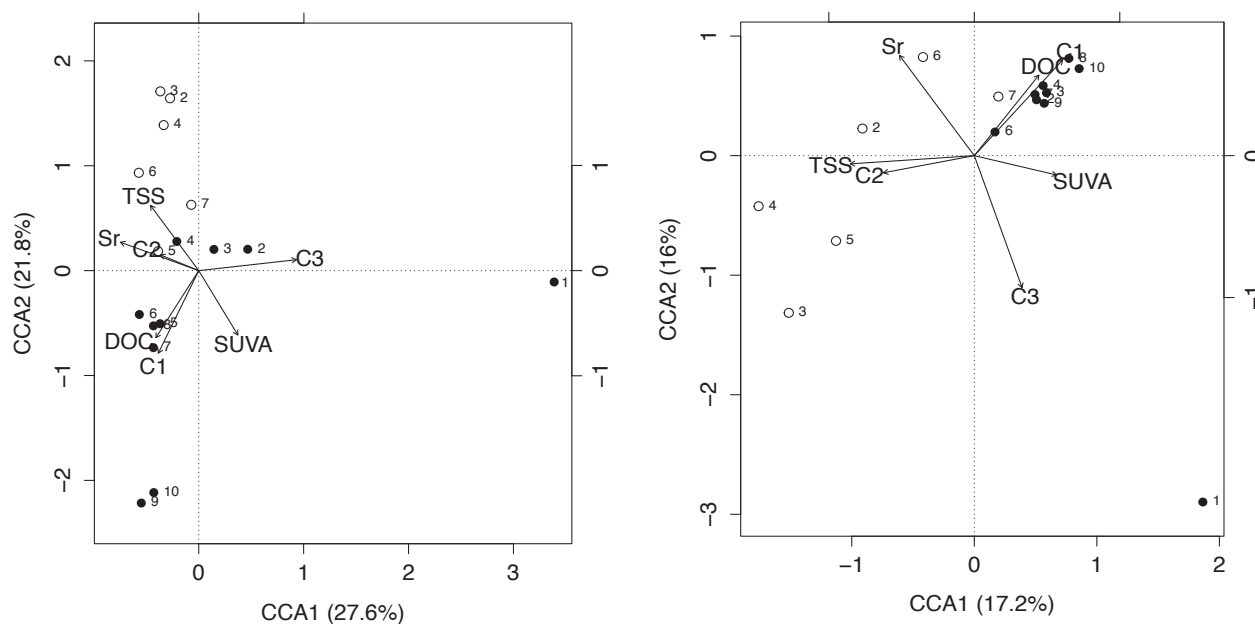


Fig 5.9: CCA analysis between bacterial community and environmental factors bacterial community for the PA (left) and FL fractions (right) for stream water samples S4 (filled circle) and S10 (open circle).

5.2. Impact of overland flow on particle-attached and free-living fractions during a flood event in an upland tropical catchment

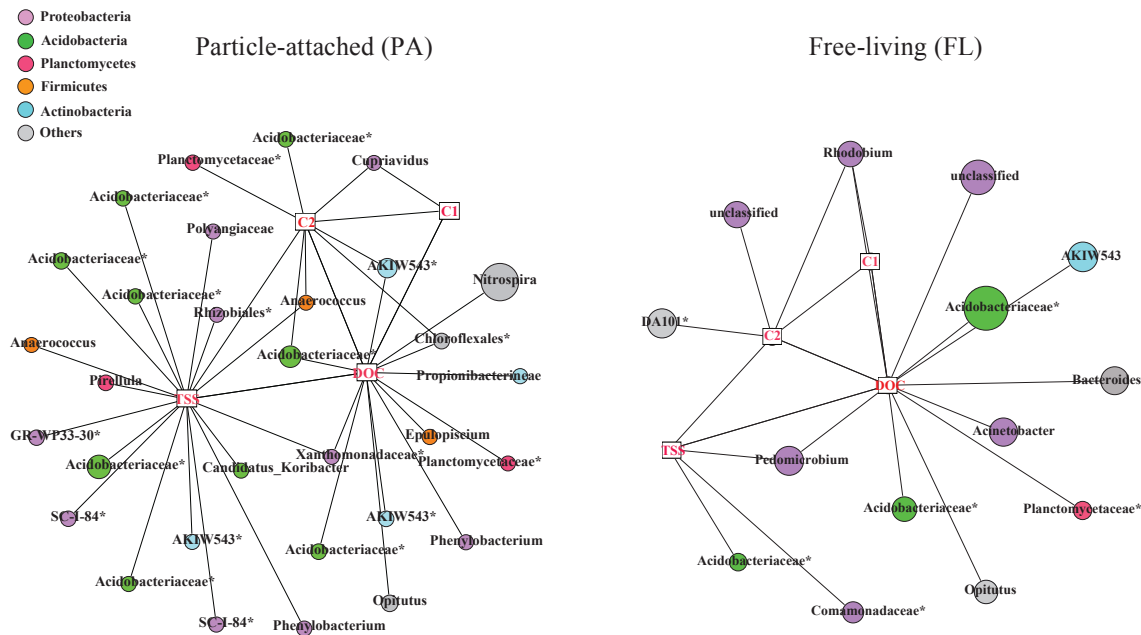


Fig 5.10: Network analysis of relationship between environmental factors: Dissolved organic carbon (DOC), the humic component (C1, C2), total suspended sediment (TSS) and OTUs. The color of node based on the bacterial phylum. The label shown in node relates genus level. The size of node relates to their number of sequences. * Unidentified OTUs belonging to family or order level.

Moreover, a cooccurrence network analysis was performed for stream water at S4 to assess the temporal effects on relationship between environmental factors and bacterial community during the flood (Fig 5.10). The results revealed DOC was significantly associated with the humic components (C1 and C2) and TSS. These factors were significant correlated with more PA-OTUs than FL-OTUs. For PA fractions, DOC concentration was significantly correlated with 13 OTUs which belong to *Nitrospira*, *Propionibacterineae*, *Acidobacteriaceae*, *Xanthomonadaceae*, *Opiritutus*, *Phenybacterium*, *AKIW543*, *Epulopiscium*, *Plantomycetaceae*, *Chloroflexales*. The genus of *Nitrospira* and *Acidobacteriaceae* were the high abundant OTUs amongst OTUs related to DOC concentration. The component C2 was correlated with 7 OTUs including *Anaerococcus*, *Cupriavidus*, *Acidobacteriaceae*, *Plantomycetaceae*, *AKIW543*, *Chloroflexales*. TSS was correlated with 17 OTUs with 5 OTUs classified as *Acidobacteriaceae* and the remaining OTUs belonged to *SC-I-84*, *Phenybacterium*, *AKIW543*, *Priellula*, *Anaerococcus*, *Rhizobiales*, *Candidatus_Koribacter*, *GR-WP-33-30*.

For the FL fraction, DOC concentration was significantly correlated with 11 OTUs classified as *Acidobacteriaceae*, *Pedomicrobium*, *Bacteroides*, *AKIW543*, *Opiritutus*, *Plantomycetaceae*. The humic component C2 was correlated with 3 OTUs including genus of *Rhodobium* and OTU belong to *Gamma-Proteobacteria*. Meanwhile TSS was correlated with 3 OTUs including genus of *Comamonadaceae*, *Pedomicrobium*, *Acidobacteriaceae*. The most abundance OTU in the network was *Acidobacteriaceae* that correlated with DOC concentration (Fig 5.10).

5.2.4. Discussion

Overland flow at a flood event significantly contributes stream water with allochthonous organic carbon.

Our results showed that DOC and TSS increased with increasing flood discharge and overland flow during the course of flood, suggesting an increase in terrestrial inflow during the course of the flood. TSS and runoff were previously found to increase during storm events and to introduce allochthonous material into receiving water bodies (Ulrich et al., 2016). Similarly, a significant increase in DOC was also observed in the Hunter river and its tributaries in Australia, after a major flood event that impacted downstream sites for several months (Carney et al., 2015). Besemer et al. (2005) also found that flood events increased the amount of DOM, probably as a consequence of inputs from previously disconnected pools and increased terrestrial inflows. In our study, FI ranged from 1.11 – 1.37 indicating that the source of DOC was from terrestrial plants and soil organic carbon (Fellman et al., 2010). The decrease of FI with increasing DOC concentration during the flood recession indicated that more allochthonous DOC was exported from overland flow. Moreover, the three components of PARAFAC (C1-C3) also provide evidence for terrestrial source of DOC. The component C1 and C2 were found characteristic of humic-like substances (Fellman et al., 2010; Yamashita et al., 2011).

Compared to before the flood event, a decrease of $SUVA_{254}$ and of molecular weight (increase of S_r) at high flow upstream (S4) indicated that DOC released during the flood event was less aromatic and of lower molecular weight, but more humified. This has also been observed during a storm events in a peatland catchment in North Wales, UK (Austnes et al., 2009). Fellman et al. (2008) showed that a high $SUVA$ is associated with lower bioavailability. Biodegradation is known to increase the of aromaticity of DOM (Kalbitz, 2003). Therefore, the DOC released at S4 during the flood event was found less degraded, less aromatic and more bioavailable. This suggested soil water comprised more fresh, less degraded DOC than stream water at base flow (Evans et al., 2007). Contrasting with our results, some studies reported that the aromatic contents of DOM ($SUVA_{254}$) increased with storm discharge (Hood et al., 2006; Fellman et al., 2009; Inamdar et al., 2011a). This pattern was found downstream (S10) in our study but the value of $SUVA_{254}$ at S10 was still lower than at the S4 sampling site.

Overland flow drives bacterial community structure during the flood event

Floods triggered by single heavy precipitation events are considered to be pulse events in stream ecosystems (Lake, 2000). Bacteria in stream water may be disturbed by a random and stochastic (or regional) factors caused by flood events or by deterministic process (local factors) if the intensity of flooding becomes strong enough (Lepori and Malmqvist, 2009; Febria et al., 2012).

Overland flow can bring a mixture of particulate matter and bacterial community from hillslopes to the stream during a flood. This corresponds to the observed increase in richness of bacteria during the flood studied here as compared to base flow. Due to the short water residence times in the stream during

this flood event, dispersal was most likely the dominant mechanism driving bacterial community with the advection of a large number of cells into the stream from the slopes (Crump et al., 2003; Lindstrom and Bergstrom, 2005). Crump et al. (2007) indicated that average water residence times in streams were shorter than community growth rate during high flow, implying that streams act as a conduit to transfer communities that are dominated by the bacterial community washing from upslope environment. The mixing of the bacterial community from upland with the stream water can shift structure of initial community in the water of stream (Crump et al., 2012). Adams et al. (2014) found that the high dispersal rate periodically disrupts the stable community composition during large storm events, resulting in a similar bacterial community between inlet and outlet. Our study indicated that more bacteria originating from PA fraction of the overland flow entered the stream water during the flood (Fig 5.7). This is also supported by the fact that the structure of PA fraction of stream water was closer to that of overland flow (S8) compared to the FL fraction.

This suggested that during the flood recession stream bacterial community was driven by the dispersal of the PA bacteria originating from soil. Besemer et al. (2005) in temperate river-floodplain system also indicated that the PA bacterial fraction was probably more strongly impacted by hydrology and changes in environmental conditions such as the inflow of terrestrial organic matter than was the FL fraction. As the inflow of bacteria attached to particles from the terrestrial environment during the flood event might survive in the aquatic system for some time their impact on the community could be more than just transient (Besemer et al., 2005). As an example, it could have a dramatic increase of fish mortality several months after the flood event (Carney et al. 2015).

Our study also found that the PA fraction community structure showed similarities between upstream (S4), downstream (S10) waters and overland flow (S8). Both S4 and S10 were surrounded by teak plantations on the basin slopes, which underline this importance of vicinal LU on the stream bacterial diversity (Le et al., 2018). The similarity of PA fractions between S4 and S10 suggests the PA fraction was carried from soils under teak plantations via overland flow into the stream during the flood event. However, a stronger effect of overland flow occurred upstream (S4) than downstream (S10) because higher allochthonous DOC and ratios of OTU exported from overland flow into stream water were found at S4. Although the stream received more overland flow during the flood, this mixture of flow from upland and ground water may rapidly wash out from the stations by high rate of discharge during the flood. This reduces the similarity in community composition from upland or contributing environments to that of downstream ones as the flood recedes (Crump et al., 2007; Adams et al., 2014). This mechanism probably explains why the ratio of OTUs from overland flow in the stream water drastically decreased at the end of the flood.

Relationship between bacterial community structure and environmental variables

Our study focused on finding relationships between bacterial community structure (for the PA and FL fractions) and environmental factors including TSS, DOC and its optical characteristic in upland catchment during a course of flood. A CCA analysis was conducted between environmental variables and the PA and FL fractions separately to understand which factors significantly controlled the different PA and FL fractions during a course of flood. We found that TSS was one of the most important factors driving bacterial community composition for both PA and FL combined at different sites during the flood. When assessing the sediment sources during this flood event, Evrard et al. (2016) found that at the upstream station (S4), re-suspended subsurface material on the riverbed was re-mobilized at the first stage of flood and that this decreased with increasing flow inputs of surface material originating from hillslopes cropland during peak flow and flood recession stage. In contrast, the source of particle matter of S10 was mainly from re-suspended subsurface sources that was deposited on the streambed (Evrard et al., 2016). This suggests that the relative contribution of re-suspended subsurface and eroded surface sources to river sediments during floods may be significant in controlling the structure of the bacterial communities from both PA and FL fractions in the flood. The storm water influx and the associated sediments in free-flowing water may be the principal factor influencing bacterial community structure during the flood (Ulrich et al., 2016). Luef et al. (2007) also proposed that the PA fraction was more affected by the quality of particle rather than by the hydrology of the system. Smaller particles are thought to be colonized more densely by bacteria than are larger particles (Kondratieff and Simmons, 1985). Tang et al. (2017) also indicated that turbidity or total suspended solid (an indicator of sediment resuspension and phytodetritus) was one of the main factors controlling the structure of bacterial communities from the PA fraction. Therefore, the sources of particles and pathways of water play a key role in driving the bacterial community.

Moreover, DOC and the humic components were more related to temporal shift in the PA bacterial community than the FL fraction. This suggested the PA fraction was more correlated with the upland terrestrial flow than the FL fraction during the flood event. This pattern was supported by network analysis which shown that more PA-OTUs was significant correlated with DOC, the humic components and TSS than FL – OTUs. Similarly, DOC and the humic components were found as more important role in PA co-occurrence networking than FL fraction in this catchment at base flow in the wet season (Le et al., 2018). This suggested the stream bacterial community was driven by upland soil through hydrological connection at the base flow(Le et al., 2018) and overland flow during the flood event.

The genus of *Nitrospira* that was found predominant in the PA fraction was found related DOC concentration in stream water during the flood event. *Nitrospira* as an aerobic nitrite-oxidizing bacterium in the nitrification process play a key role in soil N dynamic (Daims et al., 2015; Le Roux et al., 2016) and linked to a substantial metabolic versatility such as utilization of organic compounds, urea

degradation (Daims et al., 2015; Gruber-Dorninger et al., 2015; Koch et al., 2015). This suggests that the loss of the taxa in overland flow into stream not only results in a shift in stream metabolic functions but also affect the functional groups in upland soils during the flood. Such consequences deserve deeper investigation to properly assess the impact of flood event on functional diversity in soils.

5.2.5. Conclusion

During the flood event, stream water was dominated by terrestrial flow and its associated characteristics including allochthonous DOC and bacterial community attached to particles. The number of PA taxa originating from overland flow increased in stream water as the flood progressed and was higher than that of FL taxa. This study suggests that the PA fraction depends more on eroded particle material flow from hillslope terrestrial runoff than FL fraction. The study provides fundamental insights into understanding how the transportation of allochthonous DOC and the soil bacterial community, via overland flow, impact the stream bacterial community during a flood event. Although the stream flow was rapidly wash out during the flood, the connection between terrestrial runoff and stream was still found. Thereby its effects on soil functions and stream bacterial structure should be considered on a long-term perspective, especially for catchment management.

Supplementary information

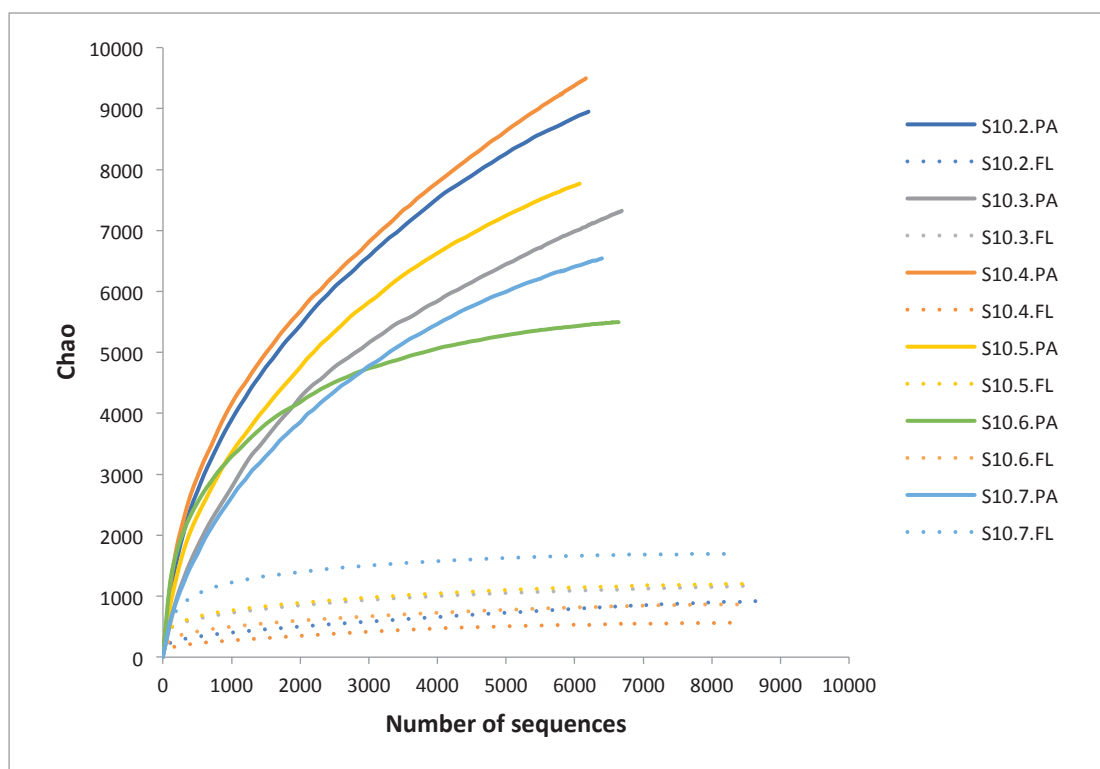
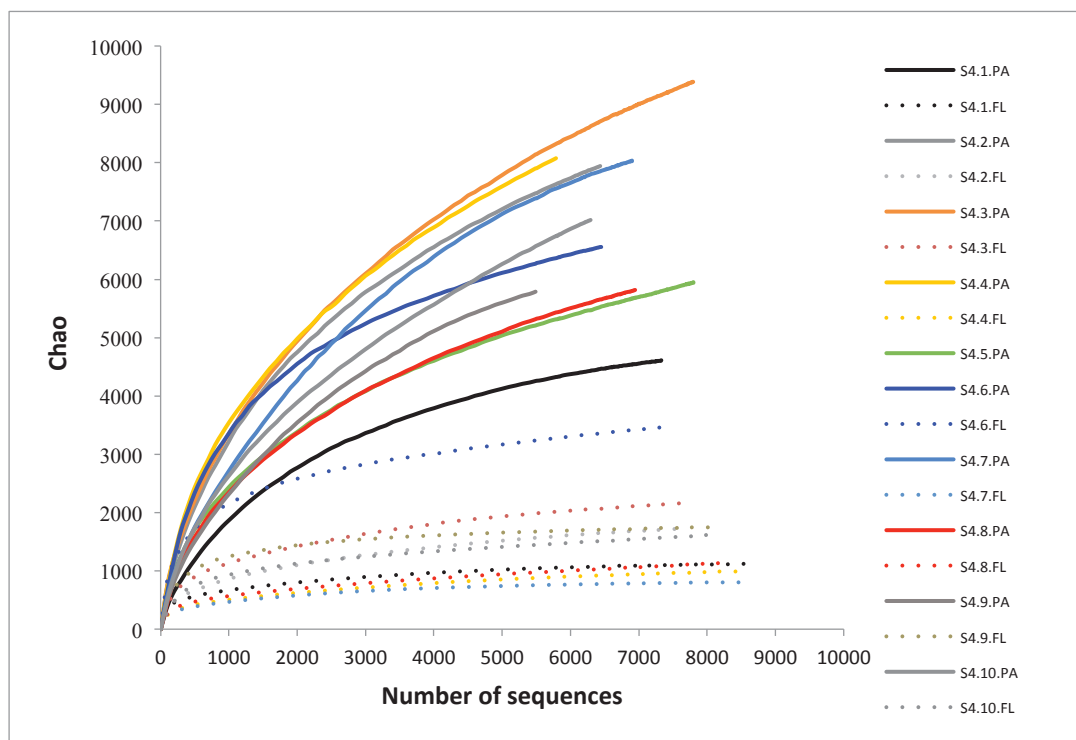


Fig S 5.6: Rarefaction curves of Chao1 richness estimates for all samples at S4 and S10 during the flood.

5.2. Impact of overland flow on particle-attached and free-living fractions during a flood event in an upland tropical catchment

Table S 5.5: The optical characteristic of dissolved organic carbon (DOC) in flood event. Sr: the inverse of molecular weight, SUVA₂₅₄: the aromaticity of DOC, HIX: humification, FI: fluorescence, C1, C2: humic component, C3: tryptophan-like fluorescence

	Sr	SUVA₂₅₄	HIX	FI	C1	C2	C3
S4-1	0.58	7.15	0.18	1.22	0.17	0.18	5.00
S4-2	0.95	6.57	0.42	1.37	0.28	0.18	0.08
S4-3	1.02	7.09	0.42	1.28	0.29	0.18	0.09
S4-4	0.90	6.12	0.43	1.25	0.31	0.21	0.15
S4-5	0.90	6.28	0.40	1.23	0.31	0.21	0.37
S4-6	0.88	6.94	0.42	1.22	0.36	0.26	0.18
S4-7	1.00	4.91	0.40	1.20	0.32	0.22	0.16
S4-8	0.85	5.09	0.46	1.18	0.36	0.25	0.07
S4-9	0.82	6.88	0.54	1.20	0.36	0.24	0.07
S4-10	0.91	7.69	0.46	1.27	0.35	0.24	0.10
S8-2	0.98	6.67	0.39	1.12	0.31	0.18	0.09
S8-8	0.88	3.59	0.43	1.12	0.34	0.25	0.09
S10-2	1.05	4.97	0.36	1.10	0.19	0.19	0.10
S10-3	0.85	5.14	0.27	1.20	0.16	0.28	1.52
S10-4	1.00	5.16	0.37	1.11	0.16	0.34	0.23
S10-5	0.89	6.18	0.29	1.40	0.13	0.48	0.95
S10-6	0.95	5.86	0.39	1.12	0.24	0.30	0.08
S10-7	0.99	5.27	0.40	1.15	0.26	0.19	0.09

Table S 5.6: Estimated Chao 1 Richness and Shannon diversity of bacterial community during the flood at S4 and S10 station for particle attached fraction (PA), and free-living (FL) fraction.

	Chao 1		Shannon	
	PA	FL	PA	FL
S4-1	4250	1044	6.40	6.14
S4-2	7503	1568	6.82	4.77
S4-3	8120	1990	7.17	5.43
S4-4	7900	883	6.98	5.24
S4-5	5241	-	6.22	-
S4-6	6266	1948	7.09	6.79
S4-7	7393	755	6.56	5.60
S4-8	5325	971	6.43	5.70
S4-9	5788	1676	6.15	6.60
S4-10	6573	1445	6.39	5.04
S10-2	8573	766	7.40	5.58
S10-3	6730	1072	5.88	6.12
S10-4	9021	524	7.29	4.78
S10-5	7513	1126	7.15	6.32
S10-6	5360	801	7.51	5.87
S10-7	6221	1647	6.89	6.53

5.2. Impact of overland flow on particle-attached and free-living fractions during a flood event in an upland tropical catchment

Table S 5.7: Environmental factors and particle-attached (PA), and Free-living (FL) fraction structure during a flood event. Stars indicate the level of significance of the P-value (*=0.01; **=0.001; ***=0)

Fractions	CCA1		CCA2		R ²		P value	
	PA	FL	PA	FL	PA	FL	PA	FL
DOC	-0.57	0.61	-0.81	0.79	0.49	0.51	0.016*	0.013*
Sr	-0.94	-0.58	0.33	0.81	0.6	0.76	0.045	0.003**
SUVA	0.58	0.97	-0.81	-0.23	0.46	0.33	0.008**	0.08
C1	-0.46	0.66	-0.88	0.74	0.64	0.81	0.001***	0.001***
C2	-0.93	-0.98	0.36	-0.18	0.14	0.39	0.280	0.092
C3	0.99	0.33	0.06	-0.94	0.83	0.97	0.058	0.001***
TSS	-0.64	-0.99	0.76	-0.06	0.55	0.72	0.003**	0.001***

CHAPTER 6

EFFECT OF OVERLAND FLOW FROM DIFFERENT LAND USE ON AQUATIC BACTERIAL DIVERSITY AND FUNCTIONING IN *MICROCOSM SCALE*

This chapter presents preliminary results from the microcosm experiment that will be further analyzed for a subsequent publication.

6.1. Introduction

The results from the field investigation described in the previous chapter 5.1 confirmed that the stream bacterial community was driven by LU change in the uplands through hydrological connectivity in the catchment (Le et al., 2018). Terrestrial runoff, especially from TWO, exported DOC and specific soil bacteria into stream at high flow. A strong relationship between DOC and stream bacterial community was found throughout this thesis. To support the result from field investigation, the controlled mesocosm/microcosm experiments were conducted to assess how the aquatic bacterial community responded by directly adding overland flow from different LU. While the field investigation gave a comprehensive vision at the catchment scale about the effects of LU change on stream bacterial community, the incubation experiments gave a small and detailed vision about the response of stream bacterial community to terrestrial runoffs from different LU and agricultural practices. Moreover, with mesocosm and microcosm experiment in this work, I examined which types of soil bacterial community entered stream water and how these soil communities may adapt to stream local conditions and compete with local resident communities during the incubation. The effect of dispersal mechanism and seasonal variation of hydrology conditions were thus removed in these experiments.

The results from the mesocosm experiment (Chapter 3) showed that the community structure in reservoir water with runoff from soil amended with compost, biochar and vermi-compost changed over the course of the incubation. This indicates that the addition of surface runoff into reservoir resulted in changes in bacterial structure. This study also showed that the richness and the evenness of bacteria in mesocosms receiving runoff water from soil amended with organic fertilizers decreased in parallel to an increase in DOC concentration. This suggested there was a specialization of species when both carbon sources were present. In general, the mesocosm experiment showed the shift of aquatic bacterial diversity and structure when adding terrestrial runoff from soil amending with different organic fertilizers (Le et al., 2016). As detailed below this result was confirmed and developed with microcosm experiments when considering the response of stream bacterial community to terrestrial runoff from different LU in the Houay Pano catchment for a short term (96 hours) response. Therefore, both mesocosm and microcosm experiments allow assessing the effect of terrestrial runoff from both agricultural practices (applying organic fertilizers) and LU changes on aquatic bacterial community.

Moreover, microcosm experiment was more related and supported the results from *in situ* investigation in this catchment.

6.2. Material and methods

Soil sampling

20 soil cores (0-10 cm deep) along a 25m-transect were collected and pooled from each LU: teak with understory (TW) and teak without understory (TWO), 2 years fallow after 7 years rotating cultures (2YF), 8 years fallow after 7 years rotating cultures (8YF) from the Houay Pano catchment, Laos.

Microcosm experiments

One hundred and fifty (150) gram soil from each LU: teak with (TW) and without understory (TWO), 2 years fallow after 7 years rotating cultures (2YF), 8 years fallow after 7 years rotating cultures (8YF) was collected. Each soil was washed with 1 L water collected from the upper stream station (S4) and was shaken for 15 min and then filtered at 250 μm . From each ex-filtrate, 800 mL were collected and re-filtered through a 63 μm nylon filter. For each LU, the prepared soil overland flow was divided into 4 aliquots of 200mL.

Twenty plastic bottles of 1.5 L volume including 4 replicates of each of the 4 LU plus 4 replicate stream water controls were filled with 1 L stream water collected from S4. Two hundred (200) mL of overland flow water was added into 1 L water stream, except for the control microcosms (stream water only). All of the microcosms were incubated for 96 hours. Microcosms were mixed daily and samples were collected for DOC concentration at 0h, 24h, 48h, 72h and 96h of the incubation and bacterial diversity at 0h, 48h, and 96h, metabolic capacity at 0h and 96h of the incubation experiment.

DOC concentration measurement

DOC concentration was measured on a Shimadzu Total Organic Carbon (TOC) V_{CPH} analyzer following the method described in Rochelle-Newall et al. (2011). DOC concentration is expressed as mg of organic C per liter of water (mg L^{-1}).

Estimation of bacterial diversity and metabolic capacity

DNA extraction and 16S RNA sequences were conducted to measure bacterial community structure and diversity as described in the previous chapters. Similarly, Biolog[®] Ecoplates were used to identify the C utilization of the bacterial community as described above.

6.3. Results and discussion

DOC concentration

The addition of overland flow from different LU increased DOC concentration by a factor of ten in the treatment microcosms compared to control microcosm (stream water only). DOC concentration gradually decreased in all microcosms over the 96h incubation (Fig 6.1). The organic carbon in the overland flow was highly bioavailable to the aquatic bacterial communities with removal rates of 31.5 % for 8YF microcosm, 12.2% for TWO, 7.5 % for TW and 6.67% for 2YF observed. This is comparable to the DOC removal rate of 12% to 27% observed a series of lake water microcosm experiments subject to addition of leachate from soils amended with different organic fertilizers during 72h incubation (Pommier et al., 2014).

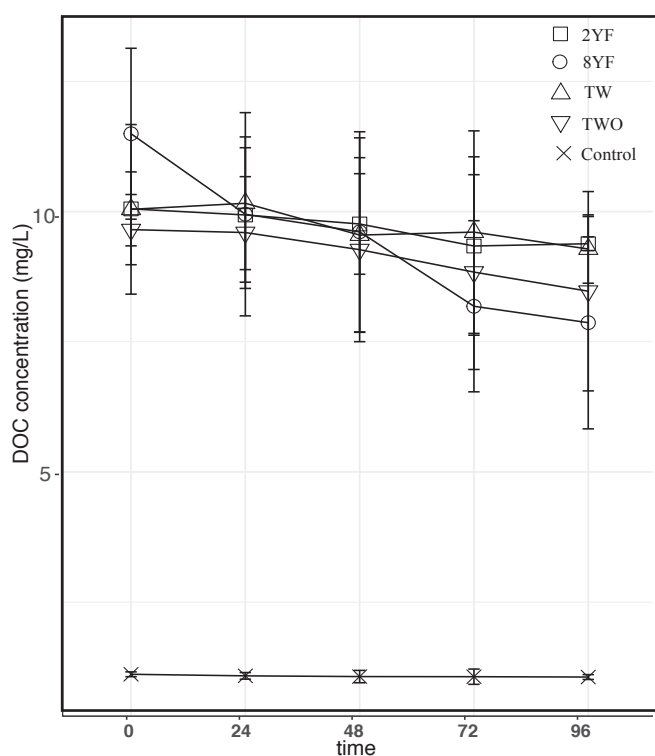


Fig 6.1: Dissolved organic carbon (DOC) concentration (mg L^{-1}) in treatment (TW, TWO, 2YF and 8YF) and control microcosms during the incubation experiment

Bacterial community diversity, structure and metabolic capacity

The NMDS analysis indicated that the stream bacterial community structure shifted when adding overland flow from the different soils (ANOSIM, $R=0.65$, $P=0.001$) (Fig 6.1). The bacterial community structure changed after 4 days incubation for all treatments, especially in 2YF and TW (Fig 6.2).

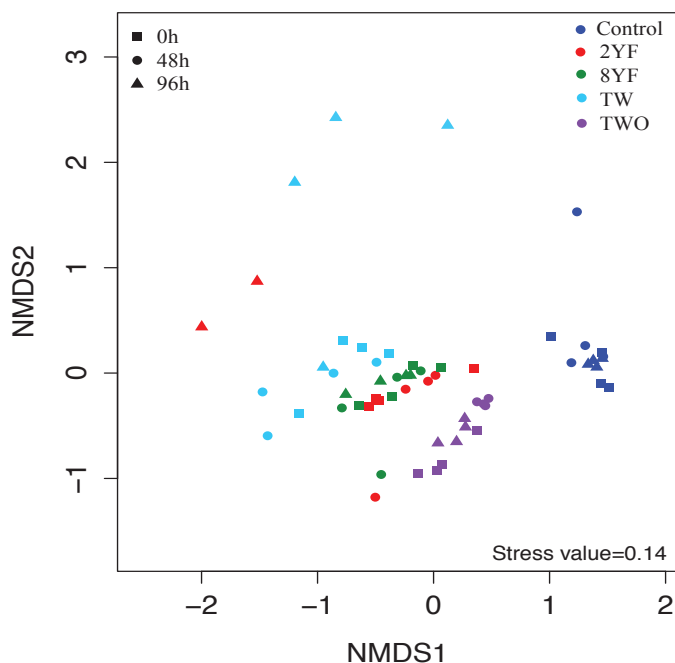


Fig 6.2: NMDS of bacterial community structure in the treatment and control microcosms under different LU and control microcosm after 0, 48 and 96h of the incubation

Similarly, the composition of the community at the phylum level changed in the microcosms with added overland flow from the different soils as compared to the control microcosm (Fig 6.3). There was an increase of *Actinobacteria*, *Acidobacteria* and *Planctomycetes* and a decrease of *Betaproteobacteria* and *Bacteroidetes* in the treatment microcosms. Noticeably the abundance of the phyla *Actinobacteria*, *Acidobacteria* and *Planctomycetes* was also higher in the soil samples under TW, TWO and UR in the field investigation. Moreover, the results from the rain simulation experiment also indicated that the group of bacteria loss from soil into runoff was mainly from the phylum of *Acidobacteria*, *Actinobacteria* (Chapter 4). This suggested that the bacterial communities belonging to these phylum were transferred from soil into the stream when overland flow from different soils was added in the stream water in microcosm experiment. The *Planctomycetes* are a widespread microbial group and are found in range of habitats such as cultivated, pasture, forest and tundra soil (Buckley et al., 2006). The *Acidobacteria* were widely distributed and more resistant to changes of nutrient resources (Fierer et al., 2007a; Jones et al., 2009).

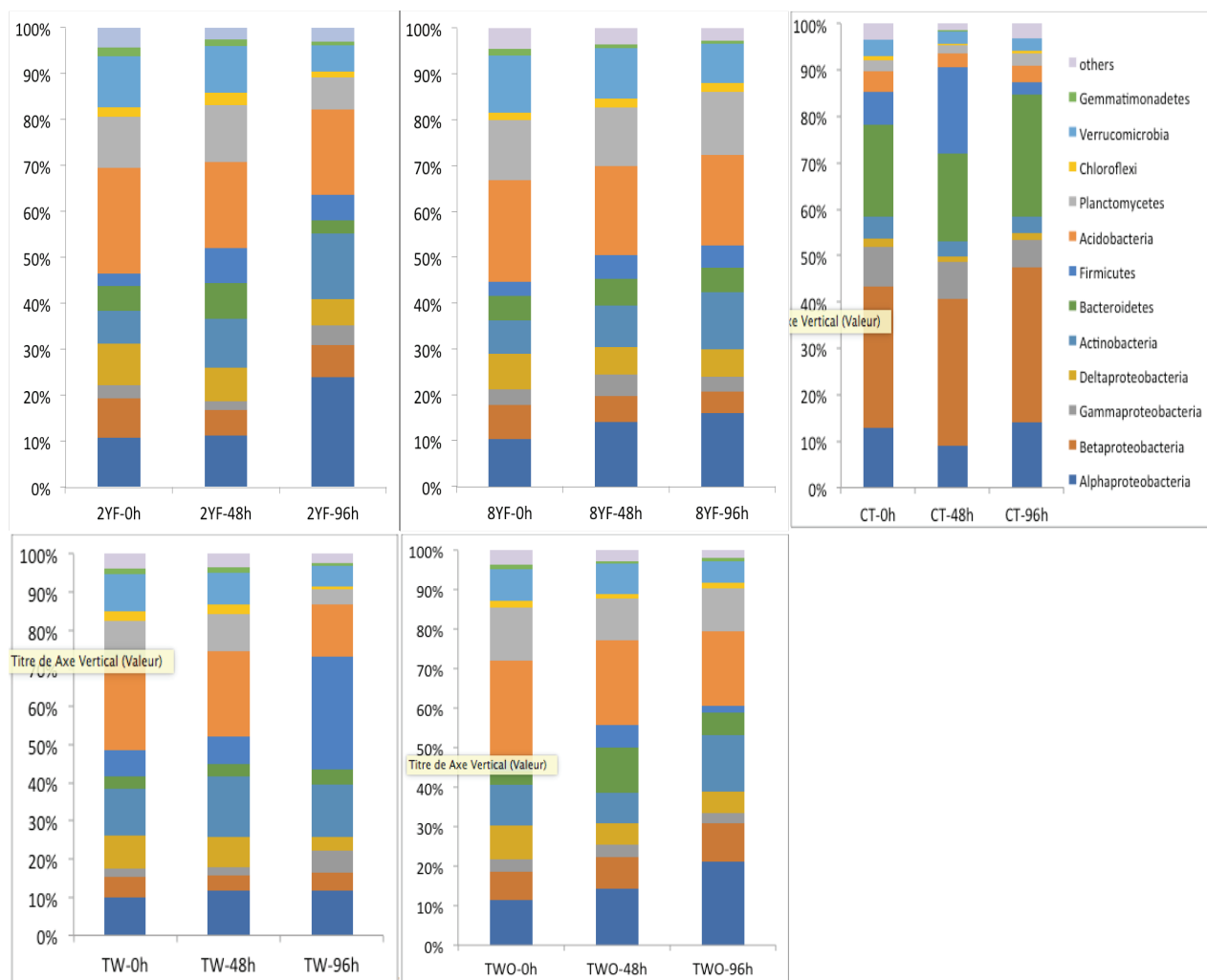


Fig 6.3: Taxonomy at the phylum level for bacterial community in the treatment and control microcosms experiment after 0, 48 and 96h of incubation.

The relationship between bacterial community structure and DOC concentration was analyzed by fitting DOC concentration to the ordination matrix of the second NMDS. The results indicated that DOC concentration was significant factor driving the bacterial community structure (*envfit*, $R=0.65$, $P=0.001$) (Fig 6.4). Thus, the addition of allochthonous C sources may increase the C availability to aquatic stream bacteria and drive the bacterial structure and diversity.

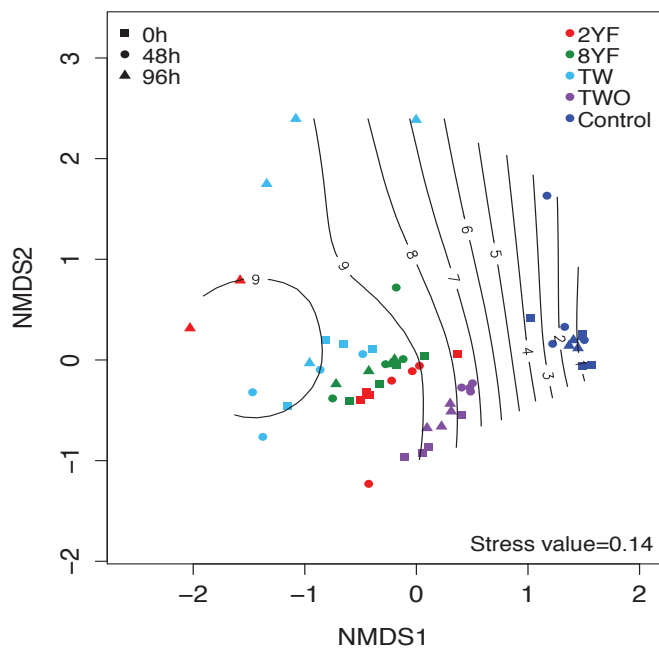


Fig 6.4: Gradient of DOC concentration was fit with bacterial community structure

Compared to the control microcosm, the richness and evenness of the bacterial community increased in the treated microcosms at the beginning of the experiment, and these values were higher in 2YF and 8YF than in the TW and TWO microcosms (Fig 6.5).

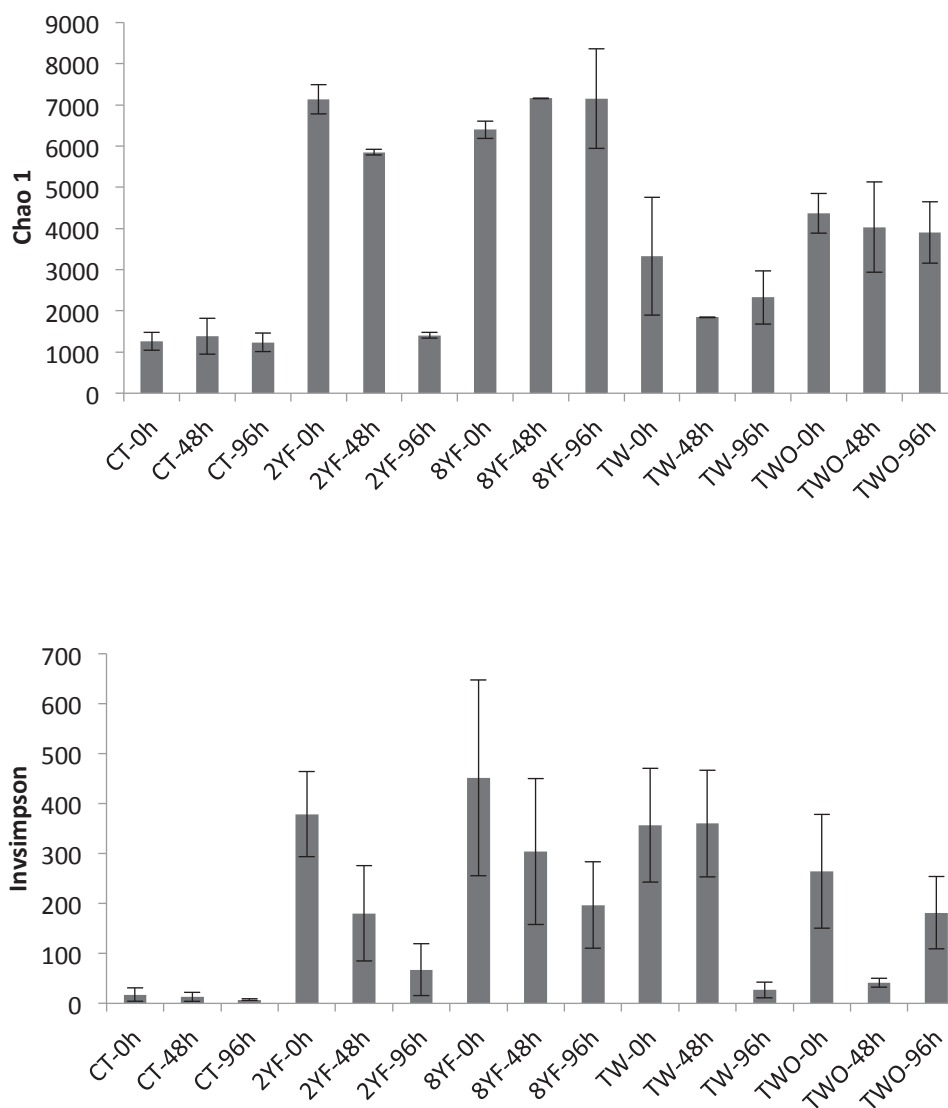


Fig 6.5: Chao 1 (richness) and Invsimpson (evenness) of the microbial community in the treatment and control microcosms after 0, 48 and 96h incubation

Similarly, carbon source utilization increased in the microcosm treatments, especially in 2YF and 8YF microcosms, compared to the control microcosm (Fig 6.6). This indicates that overland flow from different LU added different soil bacterial communities into the stream water, shifting the bacterial diversity and metabolic capacity of the stream bacterial community at the beginning of microcosm incubation. After 96h incubation, richness decreased sharply in 2YF and TW microcosms, but did not decrease in 8YF and TWO microcosms. This corresponded to a large change in community structure in the 2YF and TW microcosms between at 0 and 96 hours incubation (Fig 6.2). However, evenness decreased in all treatment microcosms after 96h incubation (Fig 6.5). This is similar to the results from the mesocosm experiments (Le et al., 2016). Although the decrease of richness and evenness was shown in almost all treated microcosms, the carbon metabolic capacity in the amended microcosms increased

for some carbon substrates, for example L-Arginine, Pyruvic acid methyl ester and 4-hydroxy benzoic acid after 4 days incubation (Fig 6.6)

This indicated a predominance of a specific bacterial community group from soil when adding overland flow to stream water over 4 days incubation. Shabarova et al. (2014) also found that longer water residence led to a decline of bacterial diversity after flooding in subsurface karst water pools. This is because microorganisms introduced to the pools during the flood faced environmental filtering toward a typical karst water community. My work also suggested some soil bacterial taxa relating to specific substrates from different LU were filtered by local environmental conditions (environmental sorting) and adapted with stream water conditions (Szekely et al., 2013; Adams et al., 2014). After 96h incubation, these specific bacterial communities adapted well to stream conditions and became more active for specific carbon degradation. Moreover, the addition of allochthonous carbon from different soils into stream may result in specialization under certain conditions in treatment microcosms (Pommier et al., 2014; Le et al., 2016). This hypothesis was tested by distinguishing between the number of specific OTUs (i.e. OTU that appeared only in the microcosm adding runoff from either fallow or in teak plantations) and the number of common OTU (i.e. OTU that appeared in both microcosms adding runoff from fallow and teak plantations) (Fig 6.7). At the beginning of incubation (0h), the number of specific OTUs was higher in 2YF and 8YF compared to TW and TWO; the pattern was more contrasted for the common OTUs. Thereafter, the number of common OTUs decreased in all treatment microcosms. Whereas, while the number of specific OTUs decreased in 2YF and TW, it increased in 8YF and TWO over the 96h incubation. Moreover, the number of specific OTUs was higher than the number of common OTUs in all treatment microcosms at the end of the 96h incubation. This suggests that some specific taxa, especially from TWO and 8YF were better adapted to stream water after 96h incubation. The ubiquity of specific taxa from TWO in soil, runoff water and stream water can be explained by better dispersion, higher adaptation and competition abilities. This result concurs with the result from the rain simulation where I observed that the highest number of specifics was lost from soil in runoff water from TWO. It can be hypothesized that because these taxa from TWO were lost more frequently than other the taxa from other LU, especially during the high flow, they have become adapted to stream water conditions. Therefore, these communities can grow better in the microcosm with stream water during 96h incubation.

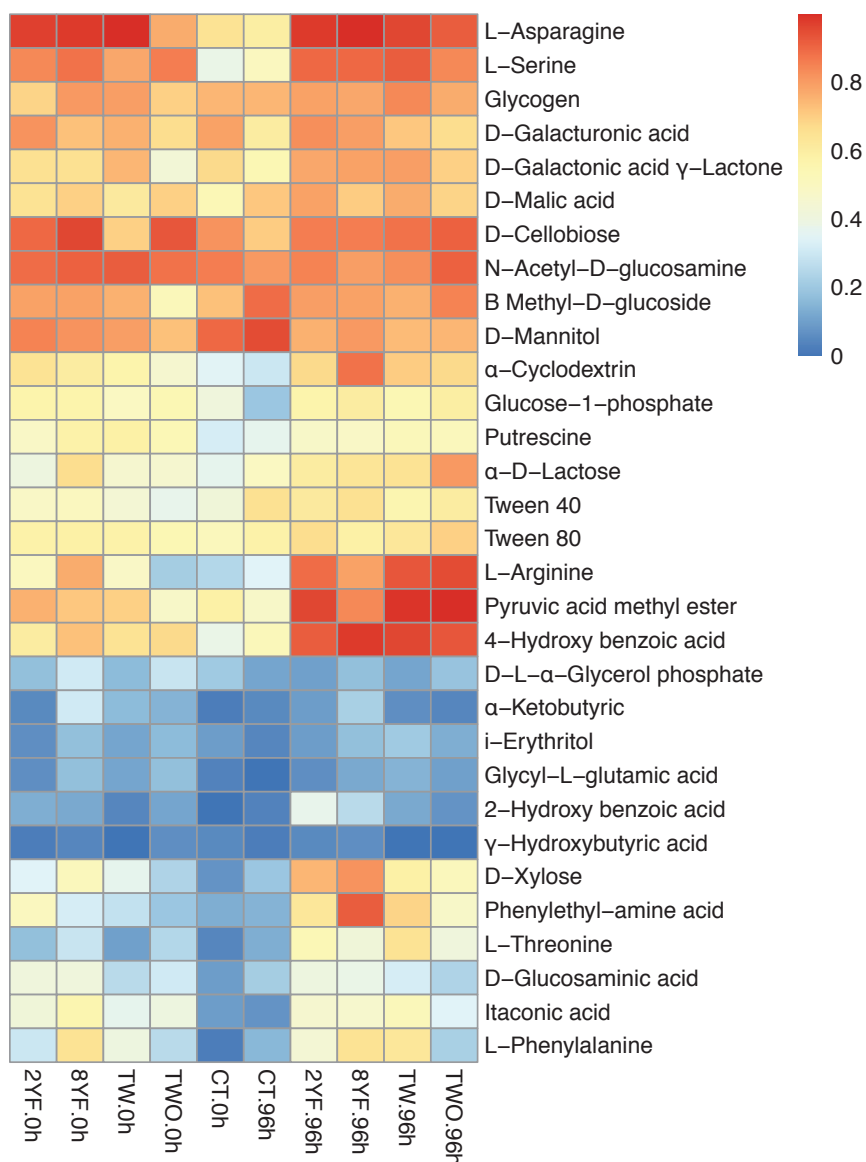


Fig 6.6: The carbon substrates utilization by Biolog® Ecoplate after a 96-h incubation in the treatment and control microcosms after 0 and 96h of incubation experiment.

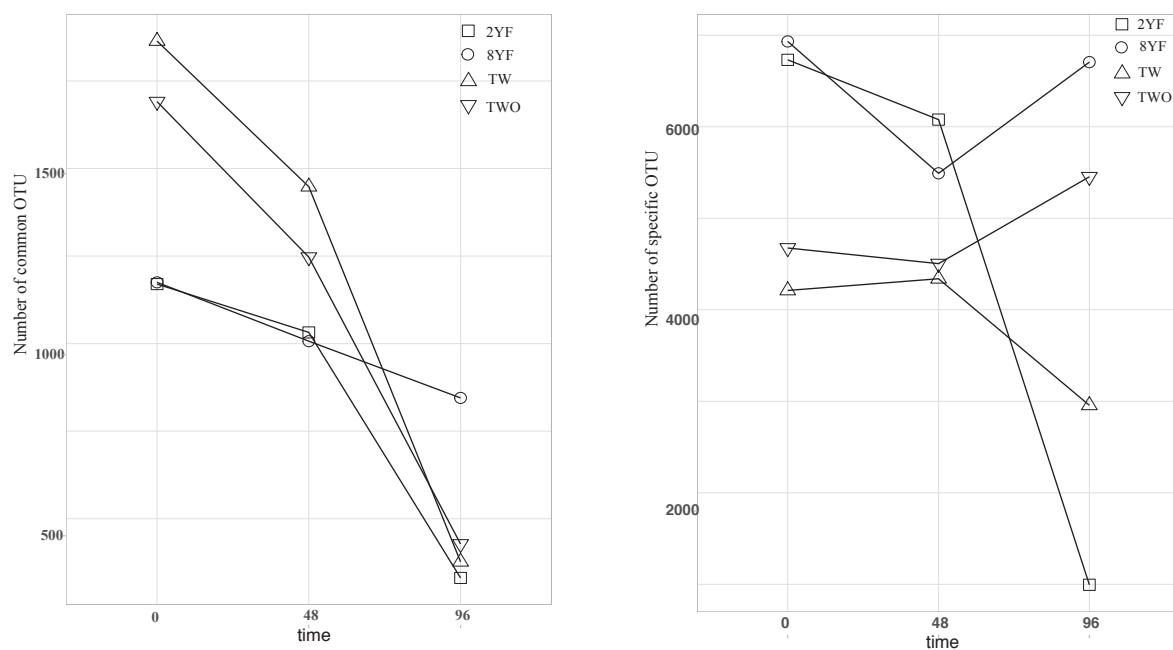


Fig 6.7: The number of specific and common OTUs in the treated microcosms after 0, 48 and 96h of incubation.

6.4. Conclusion

The microcosm experiment confirmed that the addition of overland flow into stream water caused the shift in bacterial structure and diversity. Some soil bacterial taxa relating to specific carbon substrates added into stream and developed after 4 days incubation, especially in TWO microcosm.

In summary, all of the results from the field investigations and microcosm incubations show that the bacterial community in stream water was affected by different LU, and particularly by that from TWO. It is therefore conceivable, to some extent, that a typology of aquatic bacterial assemblages could serve as a proxy of the state of agro-ecosystems and their dynamics, as initially proposed by the Teciteasy project (ANR Agrobiosphere).

CHAPTER 7

GENERAL CONCLUSION AND FUTURE PERSPECTIVE

The main objective of my thesis was to understand how LU change and different agricultural practices affect stream bacterial diversity and functioning in a tropical catchment. To fulfill this goal, I conducted my work at three different scales: mesocosm, microcosm experiments and *in situ* investigations. The field investigation was conducted to determine the changes in DOC/CDOM and the bacterial community in a stream at base flow and high flow and under real hydrological conditions, such as overland flow, in the catchment. In addition, to assess the difference of runoff from different soils under different LU, a rain simulation was conducted to collect runoff water from soils under three different LU: teak with understory (TW), teak without understory (TWO) and upland rice (UR). All of the field investigations took place in the Houay Pano catchment, Laos, as this catchment is representative of a tropical catchment in Southeast Asia, many of which are rapidly experiencing LU change from annual crops to planted forests such as teak plantations.

In addition, smaller scales mesocosm/microcosm incubation experiments were conducted to assess the direct effect of soil organic carbon and bacterial community on a stream ecosystem while removing the effects of regional factors such as hydrological conditions and seasonal variations. The mesocosm experiment was conducted in the Dong Cao catchment, Vietnam, to assess the effect of runoff from soils amended with different organic carbon sources whereas the microcosm experiment was performed to measure the effect of runoff from different LU adding directly into stream water.

The outcomes from the incubation experiments and field investigations were used to test the following three main hypotheses.

Hypothesis 1: Stream bacterial community is strongly driven by vicinal LU and its legacy

Streams are key sentinels and integrators of environmental change in the surrounding terrestrial land-landscape through transport of organic carbon, nutrient and energy (Williamson et al., 2008). The processes that affect bacterial structure in streams include regional hydrological connectivity, local environmental conditions and non-directed local processes (Freimann et al., 2015). In particular, dispersal is an important regional process that causes the movement of the bacterial community between environments, and local factors (species sorting) such as environmental conditions and biotic interactions (predation, competition, and parasitism). It may influence both the ability of new species to colonize habitats and the abundance of species within communities (Allen et al., 2011). Local environmental conditions are important for forming the structure of the bacterial community when the mechanism of species sorting dominates over dispersal during the low flow. LU change and agricultural practices can affect stream ecosystems through hydrological connectivity between soil and water including via streambed sediment in the hyporheic zone at base flow and via overland flow at high flow into stream.

Thus, the stream bacterial community may be altered due to: i) soil bacteria dispersed from terrestrial surroundings into the stream, or ii) changes in the local environmental conditions as a consequence of terrestrial nutrients and carbon inputs. In Chapter 5 (5.1), I tested the effect of LU and LU legacy on a stream bacterial community at different sites from upstream and downstream at base and high flows when the dispersal is relatively low during two periods of the annual cycle. My results show that although stream bacterial composition, structure and functioning varied with the seasonal variations of hydrological conditions, LU change and its legacy were the main determinants driving DOC/CDOM and the bacterial community along the stream. Changes in LU, particularly the transition between teak plantation and crops occurring over the previous four years led to an increase in the biodiversity of the PA bacterial community. Indeed, PA bacterial diversity was strongly associated with soil occupation (mostly teak plantations) and recent LU change from crop to teak plantations and from teak to crop in the area adjacent to the stream during the 4 years prior to sampling (2012-2015). Due to low discharge, especially in dry season when the species sorting dominated, we found that the structure of the bacterial community was more related to local site specificities (local surrounding conditions) than to stream flow path. Other work has also shown that the composition, structure and function of the bacterial community in the sediments and biofilm of a stream were influenced by LU features (Wang et al., 2011; Qu et al., 2017). At base flow, the transfer of nutrients, DOC and bacterial communities from soil into stream water occurs in the hyporheic zone (HZ) which is a transitional ecotone between surface water and ground water (Boulton et al., 1998). Indeed, streambed sediments that constitute the HZ at a certain spatial points along the stream path mainly originate from the surrounding hillslope soils that are subjected to different LU and LU legacies (Evrard et al., 2016).

I also examined the export of DOC and soil bacterial communities into stream water through a peak of overland flow during the flood event in Chapter 5 (5.2). My results show an increase of allochthonous DOC and the percentage of shared number of taxa between overland flow and stream water, especially for PA as the flood progressed. Moreover, there was an increasingly similar community structure of the PA fraction between upstream (S4) and downstream (S10) stations as the volume of overland flow from teak plantation (S8) increased. This suggests that overland flow can export particles and their associated attached bacterial community from hillslopes to stream during floods. Therefore, the mechanism driving the bacterial community during a flood event is dispersal with advection of large number of taxa into stream from upland soil (Crump et al., 2003; Lindstrom and Bergstrom, 2005).

Overall, I can validate hypothesis 1 from the results obtained during the *in situ* investigations in the Houay Pano catchment at base and high flow. The shift in stream bacterial community diversity and structure resulted not only from current LU changes but also from its legacy.

Hypothesis 2: Terrestrial runoff from different LU accelerates the transfer of the soil bacterial community into stream

The stream studied in this work received DOC and bacteria from overland flow during the flood. However, overland flow may pick up material from soils under several different LU in this catchment. To assess the differences in runoff characteristics and which types of soil bacteria and potential functions were lost into runoff water under different LU, a rain simulation experiment was conducted under three LU: TW, TWO and UR (Chapter 4). The results showed that the runoff rate, total suspended sediment (TSS), total organic carbon in TSS (TOC-TSS) were highest in runoff from soil under TWO compared to the other LU. The percentage of shared bacterial taxa between soil and runoff for PA was higher in runoff from TW and TWO than from UR. This suggested that the LU change from annual crop into teak plantation occurring in this catchment may cause more loss of bacteria into runoff water, especially for PA when it rains. Moreover, the loss of bacteria from soil to runoff was found to be highest for TWO for both PA and FL. The agricultural practice of burning understory under teak plantation (TWO) has been recently cited as being unsustainable because it causes low infiltration and high soil erosion (Ribolzi et al., 2017). The layer of surface vegetation where many attached taxa are absent resulted in the erosion of more bacteria attached to soil particles. Moreover, amongst the OTUs lost in runoff water from TWO and TW, the specific OTUs (i.e. OTU that only appeared in the runoff from TWO or TW or UR) were higher in number but were lower in sequence abundance than the common OTUs (i.e. OTU that appeared in both runoff from teak and UR). The number of specific OTUs in the runoff from TWO was higher than that in the runoff from other LU. The study presented in this thesis suggests that specific OTUs, especially from TWO were more vulnerable to loss in runoff water than the common. This is probably due to habitat specialization and it should be borne in mind that specifics are much more susceptible to changes in environmental conditions than commons (Pandit et al., 2009).

Four OTUs (OTU 16, 19, 26 and 36), denoted as common OTUs, were found in the highest abundance (more than 1%) for all soils from the three different LU. They mainly belong to the *Xanthobacteraceae* order of *Rhizobiales*. Three out of the four OTUs (OTU 16, 19 and 26) and OTU 16 were also present at a high abundance in runoff water from TWO for the PA and FL fraction respectively (from 0.5 – 2%). Only OTU 16 was highly abundant in runoff water from TW for PA (2%). Meanwhile, runoff water from UR was dominated by OTU 23, 37, 30, 71 for PA and OTU 2, 15, 21 and 79 for FL. These OTUs have abundance from 0.01-0.07% in soil of UR. They were mainly classified as belonging to the genus of *Bacillus* for PA, and *Xanthobacteraceae*, *Aurantimonas* and *Achromobacter* for FL. This suggests that terrestrial runoff from TWO caused the loss of the dominant taxa in soil. Moreover, the taxa lost belong to the *Xanthobacteraceae* order of *Rhizobiales* that was also identified from the flood event and significantly correlated with TSS in the networking analysis.

The microcosm experiment in chapter 6 also revealed that some specific soil bacteria community, related to specific carbon substrate utilisation and added to stream water in overland flow and adapted with stream water conditions, especially in treatment microcosm of TWO and 8YF.

The functional groups were also identified in soil and runoff waters under different LU by

applying FAPROTAX (Louca et al., 2016). Although only a small number of OTUs (about 10%) was referenced as functional groups due to limitations of database, the results highlighted that functional groups related to the nitrogen cycle such as nitrification, aerobic nitrite oxidation and aerobic ammonia oxidation were highly abundant in both soil and runoff waters for all LU. Moreover, these functional groups were found to be of higher abundance in runoff from TWO than from TW and UR. This indicates that the functions of soil under TWO relating to nitrogen cycles may be more degraded than under other LU. Similarly, the genus of *Nitrospira* was the most abundant OTU significantly related with DOC concentration in the flood event. The loss of this genus in the overland flow during the flood may affect nitrification which plays a key role in soil N dynamics.

To sum up, hypothesis 2 was validated with the results from the rain simulation experiment and the flood event survey. The terrestrial runoff from different LU carried different typical soil bacterial communities to the stream during rainfall. Moreover, runoff, especially from TWO, accelerated the transfer of the soil bacterial community into stream under a heavy rain and during the flood event.

Hypothesis 3: DOC of terrestrial origin controls aquatic bacterial community diversity and structure, especially for the PA fraction as compared to the FL fraction

DOC serves as an important substrate and energy source for bacteria (del Giorgio and Davis, 2003; Wei Tian, 2015a). Sources of DOC in streams include phytoplankton derived autochthonous carbon and terrestrial allochthonous DOC. Terrestrial organic carbon entering into streams can be either metabolized or deposited in the sediment of stream or can be transported downstream (Williamson et al., 2008).

In this thesis, whether in the mesocosm/microcosm experiments or during the *in situ* investigations, DOC was found to be the main factor controlling bacterial community structure in the catchment. The autochthonous vs. allochthonous ratio of carbon source may be an important structural factor influencing bacterial community composition during the incubation in mesocosm/microcosm experiment. We found increased *Proteobacteria* at higher DOC concentration after incubation of 16 days in this experiment. This is probably because the *Proteobacteria* are usually classified to comprise copiotroph bacteria that require high labile soil organic carbon to maintain their growth rates (Axelrod et al., 2002; Fierer et al., 2007a; Tian et al., 2017). This pattern was also observed in temperate river during a flood event, where Carney et al. (2015) found that *Proteobacteria* dominated during flood-affected period when bacterial communities are supported by allochthonous nutrient inputs.

For the *in situ* investigation, DOC was significantly correlated with richness and diversity of the PA fraction in stream. It also played an important role in the co-occurrence network in stream water at base and high flow (Chapter 5 5.1). Moreover, overland flow during the flood event exported a high concentration of DOC originating from terrestrial soil into stream water (Chapter 5 5.2). DOC was also a significant factor affecting bacterial community structure in runoff water from different soils collected

during the rain simulation, and in stream water during the flood event.

The concentration of DOC and the humic and fulvic acid component was found to be associated with OTUs, especially with the PA bacterial community in the sites impacted by the transition from teak to crops. Of the bacteria associated with both DOC and the transition from teak to crops, some species were found to belong to the orders *Rhizobiales* and *Rhodocyclales*, orders known for their nitrogen-fixing abilities (Pamela et al., 2012; Bao-Yu et al., 2015). The order of *Rhizobiales* was also found highly abundant in runoff from TWO in the rain simulation experiment. This result in turn highlights the potential ecological role of bacterial groups in relation to DOC runoff along the stream in the catchment.

Both the mesocosm/microcosm experiments and *in situ* investigations indicated the relationship between DOC & its characteristics on aquatic bacterial diversity and structure. But *in situ* investigations confirmed DOC was more related with PA fraction than FL fractions.

FUTURE PERSPECTIVES

The transfer of organic carbon and the soil bacterial community from soil into stream water through hydrological connectivity causes not only the loss of soil and its potential function but also induces changes in the downstream ecosystem. The stream studied here drains a catchment of 0.6 km² and flows into the Nam Khan River before its confluence with the Mekong. Therefore, the effect of LU change in this catchment can have an impact on a much wider area impacting not only the Houay Pano stream but also the Nam Khan River and the Mekong system. This highlights the importance of considering the effects of LU change in all of the countries through which the Mekong River flows. Although the study site is a small catchment (0.6 km²) with two orders tropical stream, LU change and its legacy in its uplands have significantly influenced stream bacterial community. In other catchments, the level of the effects may differ, particularly in catchments of different size and LU. For example, Harmel et al. (2010) found that *E.coli* concentration constantly decreased as catchment scale increased from field (<100ha), small catchment (100 ha to 250 km²) to river basin (>250 km²). Therefore, it will be necessary to test the conclusions presented in this thesis in catchments of different surface areas in Southeast Asia. I hypothesize that the effects of LU changes on stream water bacterial community in larger catchment is less than that in smaller catchment. This is because larger spatial scales may limit dispersal mechanisms acting on the bacterial community transferred from soil into stream water through terrestrial runoff. Moreover, it will also depend on the height of runoff water exported from different LU during rainfall events. Thus, bacterial diversity and community structure should be measured in soil samples, runoff and stream waters under different LU from a series of different catchments in Southeast Asia. The relationship between the dissimilarity of the bacterial community in different sites in a catchment and geographical distance should also be identified in order to compare the level of effect of terrestrial runoff on stream water in a range of different catchments.

The transition from annual crops to teak plantations or other commercial crops is rapidly occurring in the mountainous region of mainland Southeast Asia. For example, more than one million hectares have been converted to rubber plantation in the region and the area under rubber trees in the mountainous regions of Cambodia, Laos, Myanmar, Thailand, Vietnam and China is predicted to increase fourfold (Jefferson et al., 2014). The over-arching objective of this work was to establish a typology of bacterial assemblages relative to changes of LU and the hydro-geochemical and morphological conditions along the stream. Modeling can help to connect biogeochemical processes to specific microbial metabolic pathway (Larsen et al., 2012). It would therefore be interesting to apply modeling techniques that can identify and follow changes of bacterial diversity and functions in stream along the gradient of LU change across time and space. In this way, stream microbial communities could serve as an ecological indicator for stream water quality in the catchment subject to rapid LU change. Currently, fecal indicators are widely used to assess water quality (Jung et al., 2014; Rochelle-Newall et al., 2015). However, a single indicator or even a range of indicators is unlikely to be appropriate for every occasion and thus it is useful to tailor indicator choice to local circumstances. With the rapid disturbance of environment, the general (process oriented) microbial indicators (*i.e.* total heterotrophic bacteria) are required rather than fecal indicators (WHO, 2001). Measuring multiple bacterial indicators helps to identify of the most responsive taxa, provided preliminary information necessary for risk assessment (Paerl et al., 2003).

This study analyzed the transfer of organic carbon and the bacterial community from soil via surface runoff into the stream at base flow and during a flood event. Nevertheless, the hyporheic zone (HZ) is also an important site where exchange of water, nutrients and organic matter occurs between surface water and ground water occurs in a stream. The nutrient loading from human activities relating to LU change and agriculture practices can move from ground water into surface water through the hyporheic interface (Boulton et al., 1998). Some studies have been conducted to assess the shift of HZ microbial communities under the effect of anthropogenic nutrient loadings in temperate regions (Feris et al., 2003a; Feris et al., 2003b). But studies on how the microbial community of the HZ varies along gradients of LU change in tropical catchments are rare. In a future work, organic matter and bacterial community in the water and sediment of the HZ on a whole catchment scale could be performed to understand the effects of LU change on streams through the hydrologic exchange between hyporheic and surface habitats.

Moreover, it would be interesting to identify the relationships between diversity and functioning of the bacterial community lost from terrestrial runoff and transferred stream water. In this study, only a small part of the OTUs was referenced as functional groups by FAPROTAX (Louca et al., 2016). Metagenomics can increase the rate of gene discovery and it is a useful tool for the discovery of new genes in complex and diverse habitats (Handelsman, 2004). For a future work, metagenomics could be applied to provide more details and to reveal the wider metabolic potentials by identifying specific genes.

However, the metagenomic data is still limited in a sense that it can miss taxa due to sequences being wrongly or even not assigned, compared to 16S rRNA sequences (Poretsky et al., 2014).

In this study, DOC concentration as well as the ratio of allochthonous and autochthonous DOC were identified as being the most important factors controlling bacterial structure and composition. However, although quantifying the contribution of each source from different LU to total DOC flux is interesting, it is perhaps more important to identify the link between carbon substrates and bacterial degradation. Carbon stable isotope techniques can provide a useful tool for locating DOC sources in the landscape and following DOC transport pathways from soil to the stream, especially during the flood event (Lambert et al., 2014). Moreover, the consumption of DOC through microbial degradation is also measured with the carbon stable isotope composition ($\delta^{13}\text{C}$) (Geeraert et al., 2016)

Finally, tropical catchments are facing water resource challenges as population grows exponentially. Deforestation of upstream watersheds and LU change lead to an intensification of extreme flow events and evapotranspiration, which has influence the water balance in this area (de Paulo Rodrigues da Silva et al., 2018). My thesis has increased our understanding of the links between terrestrial environment and aquatic habitat under the effects of human activities. The result of this work emphasized that any LU change, not only current but also the legacy of LU change, and agricultural practices always have influences on stream microbial communities. This work thus contributes to the accumulation of knowledge on long-term sustainable management of soil and stream in the tropical catchment.

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Abstract

Land use (LU) change towards non-sustainable agricultural practices enhances soil degradation, erosion, and the loss of soil microbial diversity. However, the impacts of LU change on in-stream microbial community structure remain poorly understood, particularly in tropical ecosystems. Through controlled experiments and *in situ* investigations, I assessed how different LU and agricultural practices, via hydrological processes, affect the quantity and quality of stream dissolved organic matter and associated microbial community structure. The results of this work show the importance of considering both past and present LU along with hydrological processes when assessing stream microbial diversity and metabolic capacities. While the experiments in controlled conditions (micro- and mesocosms) allowed disentangling the relative importance of direct overland flow and soil community on stream bacterial structure, the *in situ* approach gave an integrated view of these processes at the basin scale. This emphasizes the need to use sustainable LU management practices if we wish to mitigate off-site impacts on downstream aquatic systems.

Résumé

L'utilisation des terres (UT) vers des pratiques agricoles non durables amplifie la dégradation et l'érosion des sols, et la perte de leur diversité microbienne. Cependant, les impacts du changement d'UT sur la structure de la communauté microbienne dans les cours d'eau adjacents restent mal compris, en particulier dans les écosystèmes tropicaux. Grâce à des expériences contrôlées et à des études *in situ*, j'ai évalué comment différentes pratiques agricoles et d'UT, via des processus hydrologiques, affectent la quantité et la qualité de la matière organique dissoute dans le cours d'eau et la structure de la communauté microbienne associée. Les résultats de ce travail montrent l'importance de considérer à la fois l'UT passée et présente avec les processus hydrologiques lors de l'évaluation de la diversité microbienne et des capacités métaboliques des cours d'eau. Alors que les expériences dans des conditions contrôlées (micro- et mésocosmes) ont permis de distinguer l'importance relative des ruissèlements d'eau de surface sur la structure bactérienne du milieu aquatique, l'approche *in situ* a permis de donner une vision intégrée de ces processus à l'échelle du bassin. Cela a mis en évidence la nécessité d'utiliser des pratiques de gestion durable d'UT si nous souhaitons atténuer les impacts sur les systèmes aquatiques en aval.