DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

Microbial decomposers diversity and litter decomposition along an altitudinal gradient in tropical and temperate stream ecosystems

Cristina Alexandra Salgado Torres

2015

DEPARTAMENTO DE CIÊNCIAS DA VIDA

FACULDADE DE CIÊNCIAS E TECNOLOGIA
UNIVERSIDADE DE COIMBRA

Microbial decomposers diversity and litter decomposition along an altitudinal gradient in tropical and temperate stream ecosystems

Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob a orientação científica do Professor Doutor Manuel Augusto Simões Graça (Universidade de Coimbra) e da Professora Doutora Andrea C. Encalada (Universidad San Francisco de Quito).

Cristina Alexandra Salgado Torres

2015

DEDICATORY

This is a special recognition for the valuable effort of

my parents and my little brother,

as well as all the people who have

shared with me memorable

life experiences during this time

Acknowledgments

The current work was carried out with the financial support of the Aquatic Ecology Laboratory (LEA) of Universidad San Francisco de Quito (USFQ) with the collaboration of the EVOTRAC project (Evolutionary and Ecological Variability in Organismal Trait Response with Altitude and Climate) and the funding of the MARE (Marine and Environmental Science Center) scholarship.

This research started thanks to the confidence that Andrea Encalada had on me the first time that I knocked her door and was possible to continue with the support and patience of Manuel Graça, who accepted me as his master student in the University of Coimbra. Thanks to let me became part of your research groups and allow me to growth as a professional and as a person. I have no words to thank you enough for your trust in me and for your invaluable lessons.

I appreciate the support of the researchers and field assistants, as well as laboratory technicians, who were involved over the different stages of this project.

I want to thank specially to Maja Celinscak who worked with me in the experiment set up and material preparation for Ecuador and Colorado sites and also collaborate with the field trips, as well as José Schreckinger, who accompanied and help me with the samples collection and data information in Ecuador. I am grateful with Patricio Aigaje who was my assistant in the field and Luis Granizo. I appreciate the collaboration of Nathaly Quiroz and Andrea Tapia, who cooperated processing the samples. Thanks to all the laboratory team for the effort before, during and after the long days monitoring the streams. To Keeley MacNeil and Carla Atkinson, who work with me in the laboratory analyses in LEA laboratory. Thanks to Diana Muñoz to cooperate with this research voluntarily.

One of the experiments could not be possible without the collaboration of Marisa Rojas, who collected, packed and transported the leaf material from Colorado to Ecuador.

An special recognition to my friend and college Andrea Landeira and also to Alberto Puime, thank you both to prepare and process the samples, without you effort this part could not be possible.

I really appreciate the patience of Veronica Ferreira while was teaching me the chemical-physical procedures in the laboratory and the statistical analyses. Special thanks to the technical support of Cristina Ducal during the chemical analyses. Thanks to Sonia Serra, Seena Saavedan and Olimpia Sobral for the logistical support in the laboratory, as well as to Dona Teresa to take care of the laboratory material when I was working in the MARE installations.

Finally, but not less important I want to thanks to Fernanda Cássio and Claudia Pascoal, who allow me to performed the molecular analyses of my thesis in the Laboratory of Biodiversity at the University of Braga (Campus of Minho). I am especially grateful with Daniela Batista, who taught me the methodological steps for the denaturing gradient gel electrophoresis, as well as the support of Sofia Duarte and Isabel Fernandes and all the laboratory team who work with me during my short time there.

This work have received valuable data from different students, technicians, researchers and PIs who have been involved in the EVOTRAC project, thanks for your help sending the information that has contribute to conclude this master thesis.

ABSTRACT

The microbial decomposition rate and species richness of aquatic fungi and bacteria were studied along an altitudinal gradient from 1600 to 3800 m.a.s.l. in Ecuador (tropical ecosystem at 0° Lat) and from 1992 to 3200 m.a.s.l. in Colorado US (temperate ecosystem at 40°N). Fine mesh bags (0.5 mm) containing native alder leaves from tropical (*Alnus acuminata* Kunth) and temperate (*Alnus incana* (L.) Moench) zones were incubated in five locations along the altitudinal gradient at each latitude and retrieved at selected sampling dates to decomposition rates and diversity of decomposers.

Decomposition rates (*k*) were faster in Colorado (0.0197 - 0.0453 range) than in Ecuador (0.0065 - 0.014 range). In Ecuador litter decomposition decreased with elevation (linear regression, $p < 0.001$; $R^2 = 0.95$), which was explained by temperature difference across sites. In Colorado litter decomposition did not change with altitude (linear regression, $p = 0.48$; $R^2 < 0.001$) and was not related to any of the measured environmental parameters. Nitrates in Colorado were ~30 fold higher than in Ecuador (11.28 vs. 0.40 µg/L; t-test, $p = 0.04$) and might be overridden the temperature effect.

Microbial decomposers diversity was estimated applying the denaturing gradient gel electrophoresis (DGGE) technique and assessing the ribosomal DNA (rDNA) for aquatic fungi (ITS2 region) and bacteria (V3 variable region). Aquatic fungi diversity in Ecuador increased with elevation (linear regression, $p = 0.03$; $R^2 = 0.83$), whereas in Colorado the maximum number of taxa peaked at middle altitudes (polynomial regression; $p = 0.7$; $R^2 = 0.57$). The three PCA axis in Ecuador explained 93% of the total variability and the number of fungal OTUs related with PO_4. In Colorado, the three PCA axis explained 92% of the total variability and taxa richness was related with CPOM standing stock, shallow waters and moderate concentrations of NO_3. The number of fungal taxa recorded in Ecuador and Colorado (13.3 and 15.2 OTUs) was not statistically different (t-test; $p = 0.2$; $t = 1.43$;

df = 8). The MDS analysis using Bray-Curtis similarity coefficient determined that fungi taxa is biogeographically distributed by latitude.

Bacteria taxa units in Ecuador and Colorado decreased with altitude (linear regression; $p = 0.0001$; $R^2 = 0.58$ and $p = 0.0004$; $R^2 = 0.99$). The higher number of OTUs along the Ecuadorian gradient was primarily correlated with the 2nd PCA axis, which in turn was correlated with CPOM standing stock, NO_3 and NO_2. In Colorado the taxa variability was negatively correlated with the first PCA axis, which was mainly correlated with temperature and NO_3 concentrations. Bacteria community exhibited a cosmopolitan distribution (MDS; stress value: 0.19).

The dissimilarity among communities along the altitudinal gradients (β diversity) was lower in Ecuador than in Colorado for aquatic fungi (ANOSIM, $p = 0.51$; Global R= 0.004 vs. $p = 0.003$; Global R= 0.54) and bacteria (ANOSIM, $p = 0.54$; Global R= -0.021 vs. $p = 0.003$; Global R= 0.51).

These results suggest that (1) litter decomposition is a temperature dependent process, which can be overridden by other factors such as nutrients in the water. (2) Species richness and the community composition of microbial decomposers may be controlled by local environmental variables, such as dissolved nutrients in water and substrate characteristics (leaf quality), which may imply that broadly patterns observed on large organisms might not affect small size organisms. Besides, latitudinal species richness tendency might be barely observed along an altitudinal gradient, considering the temperature as the mainly environmental factor driving the diversity and the taxa turnover.

Key words: microbial decomposition, temperature, latitude, altitude, OTUs.

RESUMO

A taxa de decomposição microbiana de folhas senescentes em rios e a riqueza em taxa de fungos e bactérias aquáticas foram estudados ao longo de um gradiente de altitude em dois sistemas, 1600-3800 m snm no Equador (ecossistema tropical a 0 ° Lat) e 1992-3200 m snm em Colorado EUA (ecossistema temperado a 40 ° N). Sacos de malha fina (0,5 mm) contendo folhas de amieiro nativo em cada una das zonas (Equador: *Alnus acuminata* Kunth; Colorado: *Alnus incana* (L.) Moench) foram incubadas em cinco locais ao longo do gradiente de altitude em cada latitude e recuperados em datas de amostragem selecionados para determinar as taxas de decomposição e a diversidade de decompositores.

As taxas de decomposição (*k*) foram mais rápidos em Colorado (0,0197-0,0453 gama de valores) do que no Equador (0,0065 - 0.014). No Equador a decomposição diminuiu com a elevação (regressão linear, p <0,001; R^2 = 0.95), o que foi explicado pela diferença de temperatura entre locais. Em Colorado a decomposição não mudo com a altitude (regressão linear, p = 0,48; R^2 <0,001) e não esteve ligada a nenhum dos parâmetros ambientais registrados. Os nitratos no Colorado foram ~ 30 vezes mais elevada do que no Equador (11,28 vs 0,40 mg / L; t-test, p = 0.04) podendo o seu efeito sobrepor-se ao da temperatura.

A diversidad de microrganismos decompositores foi estimada aplicando a eletroforese em gel com gradiente desnaturante (DGGE), avaliando o DNA ribossomal (rDNA) para fungos aquático (região ITS2) e bactérias (V3 região variável). A diversidade de fungos aquáticos no Equador aumentou com a elevação (regressão linear, p = 0.03; R^2 = 0.83), enquanto no Colorado o número máximo de taxa atingiu um pico em altitudes médias (regressão polinomial; p = 0.7; R^2 = 0.57). Os três primeiros eixos de uma “análise de componentes principais” (PCA) no Equador explicaram 93% da variabilidade total e o número de OTUs em fungos esteve relacionada com PO_4, que foi maior nos locais mais altos e desceu para os locais mais baixos. No Colorado, os três eixos da PCA explicaram 92% da variabilidade total e a riqueza de taxa foi relacionada com CPOM, baixa profundidade da água e concentrações moderadas de NO_3. O número de taxa para fungos registrados no Equador e no Colorado (13.3 e 15.2 OTUs) não foi

estatisticamente diferente (teste t; p = 0.2; t = 1.43; df = 8). A análise MDS usando o coeficiente de similaridade de Bray-Curtis mostrou que a identidade dos OTUs em Colorado e Equador foi diferente.

As unidades taxonómicas das bactérias no Equador e Colorado diminuiu com a altitude (regressão linear; p = 0,0001; R^2 = 0.58 e p = 0.0004; R^2 = 0.99, respetivamente). O maior número de OTUs ao longo do gradiente equatoriana foi correlacionado principalmente com o segundo eixo do PCA, que por sua vez se correlaciona com CPOM, NO_3 e NO_2. Em Colorado a variabilidade dos taxa foi negativamente correlacionada com o primeiro eixo do PCA, o qual foi correlacionado principalmente com concentrações de temperatura e NO_3. A comunidade das bactérias exibiu uma distribuição cosmopolita, i.e., não houve diferenças na identidade entre Equador e Colorado (MDS; stress value: 0.19).

A dissimilaridade entre as comunidades ao longo dos gradientes de altitude (β diversidade) foi menor no Equador do que em Colorado para fungos aquáticos (ANOSIM, p = 0.51; global R = 0.004 vs p = 0.003; global R = 0.54) e bactérias (ANOSIM, p = 0.54; R = -0.021 global vs. p = 0.003; global R = 0.51).

Estes resultados sugerem que (1) A taxa de decomposição é um processo dependente da temperatura, mas que pode ser mascarada por outros fatores, tais como nutrientes na água. (2) A riqueza de espécies e a composição da comunidade de microrganismos decompositores pode ser controlada por variáveis ambientais locais, como os nutrientes dissolvidos na água e características de substrato (qualidade das folhas), o que sugere que os padrões observados em organismos de grandes dimensões pode não afetar aos organismos de menor tamanho. Além disso, a riqueza latitudinal de espécies pode ser dificilmente observada ao longo de um gradiente de altitude, caso a temperatura seja o principal fator ambiental que controla a diversidade e o turnover de espécies, uma vez que as alterações térmicas na água são menores do que for a dela.

Palabras-chave: decomposição microbiana, temperature, latitude, altitude, OTUs.

INDEX

LIST OF TABLES

LIST OF FIGURES

LIST OF ANNEX

1. GENERAL INTRODUCTION

1.1 Latitudinal Patterns in Ecology

One of the most recognizable ecological patterns on the earth is the variation in species composition and diversity among geographic locations (Willig et al. 2003; Hawkins and Felizola 2004). These patterns, depending on the scale, are mostly controlled by geographic isolation, evolutionary history, global and local climate and species interactions (Willig et al. 2003).

Von Humboldt described higher diversity patterns at tropical latitudes and a gradual decrease towards to the poles. Subsequently, numerous examples in a wide range of taxa have been documented (Rosenzwig 1992; Rex et al. 2005; Willig 2003). For instance, in North America higher diversity of butterflies occurs at lower latitudes (Kocher and Williams 2000) and on a global scale this gradient is corroborated in gastropods, freshwater and marine fishes, amphibians, certain migrant and non-migrant birds, bats and many other mammals, as well as with plants in terrestrial ecosystems (Gaston 2000; Willig et al. 2003; Rex et al. 2005). However, there are exceptions to the latitude-richness trend. For instance, aquatic macrophytes are more diverse in the median - higher latitudes and parasite richness is not directly related with latitude (Willig et al. 2003).

Several explanations have been proposed to explain the taxa richness – latitude relationship. One of the explanations is the species-area relationship, which states that larger areas house larger number of species because of larger populations sizes and lower extinction rates (MacArthur and Wilson 1963). The terrestrial and ocean areas in the tropics have larger areas that higher latitudes and therefore most likely to harbor a larger number of taxa (Rex et al. 2005; Brown 2014). Another theory relates species richness with primary production (Waide et al. 1999; Brown 2014) because allows large populations and narrower niches (Waide et al. 1999; Mittelback et al. 2001; Schmid 2002). Other theories relate taxa richness with climatic stability (Hawkins and Felizola 2004; Brown 2014), the diversification time and biotic factors such as competition and

predation (Pianka 1966; Molles 2010). Nevertheless, the species richness distribution can be the consequences of several non-exclusive factors.

The mean and annual temperature variations differ along the latitudinal gradient. In the tropics, temperature tends to be more stable along the year compared with the higher latitudes where larger annual seasonality is recorded. Hence, tropical species are evolutionary adapted to experience narrow changes of temperature and as a consequence, these organisms might show limited dispersion ability (Janzen 1967). Thereby, temperature regimes would be determining the distribution of species because of their thermal tolerance ranges.

1.2 Altitudinal patterns of species richness

Since the temperature decreases with the latitude and with the elevation, is it could be expected that the altitudinal gradient would mirror the latitudinal pattern in biological structural and functional parameters. On the other hand, if species dispersion depends of physiological constrains (narrower thermal niche) more than geographical barriers (mountains) as Janzen proposed (1967) and temperature fluctuation in altitude do not mirror temperature fluctuations in latitude, at lower latitudes I expect a higher species replacement across altitudinal gradients than at higher latitudes (see Chapter II). Furthermore, according to the Island Biogeography theory developed by MacArthur and Wilson (1963) that predict that higher number of species are likely to inhabit in larger spatial areas; as the elevation increases, the surface area becomes narrow (Körner 2007) and as a consequence, the lower altitudes would support higher diversity than higher altitudes.

As expected, species riches in hummingbirds in the Andean mountains in South America and wild bees in the German Alps decrease with altitude (Graham et al. 2009; Hoiss et al. 2012). Nonetheless, other taxonomic groups like leaf litter ants in the Western

USA and non-endemic birds in the Hendgduam Mountains at East Asia, display a hump shaped curve, a peak of diversity in middle elevations (Sanders 2002; Wu et al. 2013).

1.3 Stream ecosystem processes at different latitudes and elevations

As the diversity increases from the poles toward to the equatorial line and from high to lower altitudes, it is plausible that temperature (and other related factors) would be pivotal variables controlling key ecosystem processes; thus biological rates might be higher near to the tropics and at lower altitudes, as predicts the metabolic theory of ecology (MTE; Brown et al. 2004). The transformation (uptake and allocation) from food resources into energy by organisms is catalyzed by enzymatic activities, which obeys to the physic laws. Thus, the rate of the reactions would depend of the kinetic energy available in the environment, meaning that higher temperatures will accelerate the metabolic rates, as is explained by the Boltzmann factor ($e^{-E/kT}$) (Brown et al. 2004).

Freshwater lotic systems, receive at least 2 billon tons of terrestrial organic and inorganic carbon inputs each year. This material is transported by streams, rivers and groundwater fluxes to the sea (Cole et al. 2007) or processed (decomposed, incorporated into living mass) *in situ*. In forested low order streams, allochthonous organic matter make up around 41-98% of energy incorporated into the aquatic food web by many microorganisms and macroinvertebrates (Fisher and Likens 1973; Abelho 2005). Once that the leaves enter into streams, their soluble compounds are released into the water (leaching), they are colonized by microorganisms such as bacteria and fungi, consumed by macroinvertebrate shredders and fragmented by physical abrasion (Hieber and Gessner 2002). High water temperatures (Suberkropp and Chauvet 1995; Fernandes et al. 2009; Geraldes et al. 2012) dissolved nutrients in streams (Gulis and Suberkropp 2003; Ferreira et al. 2006; Ferreira and Chauvet 2011a) and flow (Ferreira and Graça 2006; dos Santos Fonseca et al. 2013), enhance litter decomposition rates. In addition, intrinsic leaf features

that encompasses chemical and physical characteristics, such as toughness and defense compounds also control the rates of litter decomposition (Ferreira and Chauvet 2011b; Rezende et al. 2014).

1.4 Microbial communities as key players of organic matter processing

Studies carried out in temperate streams have determined the major decomposer of leaf litter is a group of microorganisms identified as aquatic hyphomycetes, which macerate the organic substrate and improve its palatability for other consumers, particularly macroinvertebrates (Maharning and Barlocher 1996; Graça and Canhoto 2006; Bärlocher 2010; Ferreira and Chauvet 2011b). Environmental variables such as pH, oxygen saturation, nutrient availability in the water and particularly the temperature, play a pivotal role determining rates of microbial activities and shaping species assemblages, specifically regarding aquatic hyphomycetes (Dangles et al. 2004; Medeiros et al. 2009; Duarte et al. 2013; Martínez et al. 2014).

Microcosms assays revealed that several fungi species differ in their optimal temperature for growth and sporulation, displaying optimal performance within variables ranges of temperature depending of their origin (Duarte et al. 2006; Geraldes et al. 2012; Ferreira et al. 2014). If temperature affects biological performance of aquatic fungi, it also has the capability to affect ecological functions such as litter decomposition. In fact, this assumption has been corroborated by some authors who report a positive relationship between higher loss mass rate and fungal biomass (Maharning and Barlocher 1996; Ferreira et al. 2012).

There is a succession in fungal communities through the decomposition, in which taxa specialized in lignin and cellulose are more frequent at the end of the process due to the rapid loss of easily digested compounds at the beginning of the process (Dodds 2002; Royer and Minshall 2003). In more advanced stages of decomposition, bacteria start

replacing fungi as the main decomposers of small particles of organic matter (Gessner 1997). Laboratory experiments have determined that temperature modify the aquatic fungal community composition and the litter processing can be stimulated, inhibited or affected depending of the thermal conditions tested (5-24°C) and the species identity involved in the assays (Fernandes et al. 2012; Geraldes et al. 2012; Duarte et al. 2013; Martínez et al. 2014).

Since aquatic fungi species differ in their temperature niche (Fernandes et al. 2012; Jabiol et al. 2013) and assuming that their thermal tolerance range is wider in the species from temperate than from tropical latitudes (Sridhar and Bärlocher 1993), it is likely to expect faster replacement of species along the tropical gradient than in the temperate one.

1.5 Molecular techniques

Traditionally, the identification of aquatic hyphomycetes has been performed microscopically by conidia (asexual spores) morphology recognition, which makes it a high consuming method (Maharning and Barlocher 1996). Moreover, it is plausible that many species are unable to produce spores under laboratory conditions and others have not been described in the available keys (Duarte et al. 2007; Bärlocher and Marvanová 2010). Molecular techniques has become a useful, quick and reliable tool to overcome these drawbacks, because they allow to distinguish between genotypes within a community, assessing an specific DNA region called genetic or molecular marker (Maharning and Barlocher 1996; Bärlocher 2007; Duarte et al. 2012), which is defined as a gene or non-coding segment within the genome of organisms that displays enough genetic variability (Schlötterer 2004).

Different markers have been developed for fungi and bacteria identification, based on the polymorphism of the ribosomal genes, located within the RNA sequence (rRNA) (White et al. 1990; Karp 2005; Bärlocher 2007). The nuclear Internal Transcribed Spacer

or known as ITS region is useful for fungal phylogenetic and taxonomical analysis (White et al. 1990; Bruns et al. 1991; Muyzer et al. 1993) and specifically the ITS2 region (Annex 1.a) has proved to be a suitable sequence to assess the taxa diversity in aquatic fungi (Belliveau and Bärlocher 2005; Bärlocher 2007, 2010; Duarte et al. 2007, 2010). Unlike eukaryotic organisms, the bacteria identification is assessed amplifying the variable regions from the 16S rRNA sequence (Annex 1.b), which encodes the small ribosomal subunit in prokaryotes (Muyzer et al. 1993; Karp 2005). Both molecular markers display slow mutation rates that allow the identification of taxonomical units within a microbial community (White et al. 1990; Muyzer et al. 1993).

The target sequences are amplified by the polymerase chain reaction (PCR) and then resolved in a polyacrylamide gel by electrophoresis, which allows the separation of the segments through the pores of the matrix applying electric fields. The amplified sequence are visualized as bands in the gel (Karp 2005) and they are consider as operational taxonomic unit (OTU), which are not equivalent to species in large organisms (Fuhrman 2009). The use of fingerprinting techniques, such as the denaturing gradient gel electrophoresis (DGGE), provide the genetic diversity profile of the microbial community (Muyzer and Smalla, 1998). In this technique, the amplified fragments with the same length are denatured by the chemical gradient (formamide and urea concentrations) and then each molecule gets halt when the double strand is separated according to the Guanine (G) and Citocine (C) content. It means that GC- rich sequences stop the migration in the gel at higher denaturing gradients, which remains attached by a GC tail of 40-bp added during the amplification reaction (Muyzer et al. 1993; Muyzer and Smalla 1998; Duarte et al. 2007).

Molecular techniques allow inferences of taxa richness and environmental factors in situations in which poor taxonomical expertise are limiting factors and in which there fauna and flora are poorly known. This is particularly important when dealing with

microorganisms in the environment. It is plausible that is short time molecular techniques will be common in studies addressing the identity and the role of microorganisms in the environment.

1.6 Thesis hypotheses

Here I am testing five hypothesis relating diversity, function and biogeography. The two first hypotheses relate temperature with function. If temperature controls metabolism then, within a same geographic area, litter decomposition should decrease with an increase in elevation since temperature predictably decrease with altitude (Hypothesis 1). In the same way, microbial decomposition rates are expected to be faster at lower than higher latitudes (Hypothesis 2). The other two hypotheses relate diversity with elevation. If the distribution of species richness depends of the thermal tolerance of organisms then, species diversity (α diversity) of bacterial and fungi would decrease with increasing elevation because thermal fluctuations increase with elevation (Hypothesis 3) and higher microbial diversity is expected toward to the tropics (Hypothesis 4). Finally, microbial species turnover rates would be higher in the tropical gradients (β diversity) while we expect them to be lower at temperature gradients (Hypothesis 5).

To test these previous hypotheses I run litter decomposition experiments in two latitudes: tropical (Ecuador) and temperate (United States, Colorado) systems and along an elevation gradient in both latitudes. Hypotheses 1 and 2 will be addressed in chapter 1 whereas hypotheses 3, 4 and 5 will be addressed in chapter 2.

This research is part of a large scale project called EVOTRAC (Evolutionary and Ecological Variability in Organismal Trait Response with Altitude and Climate; Poff, et al. 2010), which main goal is predict the vulnerability of the organisms associated to the freshwater systems due to the climate change using elevation gradients as a model to foresee changes in the biological processes and community structure.

2. CHAPTER I

Microbial leaf litter decomposition along altitudinal gradients in tropical and temperate streams

2.1 Introduction

The main energy source of the aquatic organisms in low order streams is the organic matter produced in the riparian zone (Vannote et al. 1980). Leaf litter material is transformed and incorporated into higher levels of the food web by the action of microbial decomposers and detritivores (Baldy et al. 1995; Gessner 1997; Graça et al. 2001). In the first stages of the decomposition, aquatic hyphomycetes colonize the leaves (conditioning) decomposing the substrate due to their exoenzymes activity and enhancing the palatability for macroinvertebrates (Graça 2001; Hieber and Gessner 2002; Bärlocher 2010). Variables such as leaf quality (Lecerf et al. 2007), nutrients dissolved in water (Gulis and Suberkropp 2004; Ferreira et al. 2006) and temperature (Boyero et al. 2011; Ferreira and Chauvet 2011b; Martínez et al. 2014) can retard or hasten the litter breakdown.

Leaves with high leaf quality (high nitrogen concentration and low lignin content) tend to decompose faster than low quality leaves (Hobbie 2005; Lecerf et al. 2007; Schindler and Gessner 2009). Since aquatic hyphomycetes are the main microbial decomposer in low order streams, their activity has being associated with the intrinsic leaf features and its influence with the decomposition rate. Aquatic fungi grow faster and reproduce earlier on leaf substrates rich in nutrients with low concentrations of polyphenolic compounds (Canhoto and Graça 1996; Ferreira et al. 2012). Likewise, tougher leaves are also more difficult to decompose by fungi (Ferreira and Chauvet 2011a).

Although nutrients in the leaves are important determining the decomposition rates, aquatic hyphomycetes are also able to uptake nutrients from the water column (Ferreira et al. 2014). Nitrogen enrichment in streams stimulates fungal activity (e.g. growth, conidia production) and accelerate the decomposition rates despite the poor quality of the substrate (Gulis and Suberkropp 2003; Taylor and Chauvet 2014). Similar observations were

reported for phosphorous (Grattan and Suberkropp 2001). Consistently, microcosms and field studies revealed that an increment of both, nitrogen and phosphorous concentrations in water, increase fungal biomass, sporulation and microbial respiration; and consequently, stimulate litter breakdown. The effect of higher levels of nutrients in the decomposition rates is more noticeable when the background conditions have lower concentrations (Ferreira and Chauvet 2011a; Grattan and Suberkropp 2001; Ferreira et al. 2014).

Temperature also affects litter decomposition under laboratory conditions (Ferreira and Chauvet 2011b; Fernandes et al. 2012) and large scale studies suggest that temperature microbial stimulation is more important at lower latitudes (Irons et al. 1994; Boyero et al. 2011). However, even when microbial activity seems to hasten leaf litter processing near to the equatorial line, others studies have reported lower microbial decomposition rates and lower fungal activity (e.g. sporulation) in the tropics compare with higher latitudes (Ferreira et al. 2012; Gonçalves et al. 2006, 2007). These results suggesting that aquatic hyphomycetes may be playing a minor role in litter processing at tropics streams in contrast with previous reports.

Since temperature decreases 6ºC every 900 m in altitude increase (Sarmiento 1986; Dudgeon 2008; NASA 2014), I may speculate that changes in altitudinal gradients in mountains could mirror changes in latitudinal gradients regarding biological diversity and ecological functional parameters, given the relationship between temperature and metabolism (Brown et al. 2004). In a recent study, Taylor and Chauvet (2014) documented an increase of microbial litter breakdown rates along a river altitudinal gradient, with higher rates in downstream sites. Authors concluded that temperature was the main environmental factor driving the decomposition. At present, it would be critical to analyze whether microbial litter breakdown decreases in a similar fashion along both latitudinal and altitudinal gradients.

Given that litter breakdown is a biological process that depends on the enzymatic

activity of microbial decomposers and knowing that the rate at which the molecules are transformed depend of the kinetic energy available, which is measured as temperature; higher metabolic rates are expected at higher temperatures due to an increment of the enzymatic transformations (Brown et al. 2004).

The objective of this study was to test whether litter breakdown in streams changes along an altitudinal gradient in lower and higher latitudes according to the metabolic theory. If temperature is the major controller of microbial decomposition, I am expecting higher decomposition rates in the tropical streams than in the temperate ones, and a decrease in litter decomposition with an increase in elevation. In order to test these hypotheses, this study was carried out in low order streams within an altitudinal gradient (from 1600 to 3800 m.a.s.l.) in two latitudes: the tropic (Ecuador) and the temperate (Colorado, USA) zones using native congeneric *Alnus* species as the substrate deployed in lotic ecosystems.

2.2 Methodology

Study sites

The hypothesis was tested in 10 first order streams located along an elevation gradient in two latitudes: Ecuador (tropical ecosystem at 0° Lat; five streams) and Colorado, United States (temperate ecosystem at 40° Lat; five streams). The tropical sites are localized in Oyacachi sub-basin at the Andean Cordillera in the Napo catchment area (Amazon Basin) within a gradient from 1645 to 3847 m.a.s.l. The temperate streams are located at the Rocky Mountains Range in the Cache Poudre Basin along an altitudinal gradient spanning from 1992 to 3200 m.a.s.l. (Table 1, Annex 2). The tree line was located at 3000 m.a.s.l. in Ecuador and the riparian vegetation was composed by herbaceous plants in the upper sites, whereas in the lower locations the streams were covered by dense tree canopy. There is a gradual increase in shrub and tree diversity along the gradient being

always higher at the tropical site compared with the temperate region. In Ecuador the vegetation in the highlands was characterized for scrublands such as *Calamagrostic sp.*and *Festuca sp.*, Andean bogs (*Azorella spp*.) and several shrubs (*Gynoxis spp.*, *Diplostephium spp.*). In the lower sites the arboreal vegetation includes species such as *Symplocos quitensis, Buddleja bullata, Alnus acuminata Kunth., Weinmannia sp.* and *Myrica pubescens.* Wider daily temperature fluctuations is registered between 3000 and 4500 m.a.s.l. (Rendón 2012). Higher rainfall was registered along the gradient. In Colorado the vegetation above the 2700 m is mainly dominated by *Picea abies*, whereas the lower sites are characterized by *Pinus spp*. and *Alnus incana*. Markedly seasonality is recorded during the year, with intermediated (< 2700) and persistent (>3000 m.a.s.l.) snow cover areas (Harrington et al. 2015). Geology in Ecuador is characterized by inceptisol type (MAGAP 2012) and by gypsiferous for Colorado (Waltz 1969).

Streams characterization

Selected streams for both latitudes were pristine or with low anthropogenic impact. For each sampling site, canopy cover was estimated with a spherical densiometer in three points of the stream within a 100 m section and then averaged to obtain the total measure for each sampling site. At each location 4 counts were made facing upstream, right, left and downstream. For tropical sites, two indices were applied to characterize the quality of the stream bed (fluvial habitat quality index; IFH; Annex 3) and the quality of the riparian vegetation (QBR; Acosta et al. 2009; Annex 4).

During the study period (see below) stream water temperature was recorded hourly for each site with submersed data loggers (Solinst Levelogger Junior Edge Water Level Loggers). Only for one tropical site E1805, the temperature was registered in situ with point measurements using a field probe (YSI 63) once at each sampling date.

For tropical streams, conductivity and pH (field probe YSI 63), dissolve oxygen

(field probe YSI 550A) as well as water discharge, were measured five times during the experiments (details below). For nutrients analysis, 150 mL of stream water were collected, filtered through fiber glass filters (47 mm ø, pore size 0.7µm; Milipore, MA, USA), transported in an ice chest to the laboratory, and frozen at -20° C until being processed. Phosphorus was determined using the ascorbic acid method and read at 885 nm (Algilent Technologies, Cary 60 UV-Vis). Nitrates were measured with the cadmium reduction method (detection limit: 0.3 to 30.0 mg/L NO_3^-N), nitrites were determined with the diazotization method (detection limit: 0.002 to 0.300 mg/L NO_2^-N), sulfates by the SulfaVer 4 method (detection limit: 2 to 7 mg/L SO_4^{2-}) and ammonia measurements were performed by salicylate method (detection limit: 0.01 to 0.50 mg/L NH_3-N), all of them using a Hatch kit (Hatch Company).

In the temperate streams, all physic-chemical measurements were performed only once during the experiment: conductivity and pH (field probe YSI30), dissolve oxygen (field probe YSI proODO) and discharge. For the nutrients analysis, water was collected in the same fashion than in the tropical sites and once in the laboratory water nitrates were measured using a Dionex 1100 ion chromotography and phosphates were analyzed by the ascorbic acid method using the Asoria2 Segmented flow analyzer (Astoria-Pacific, Clackamas, OR).

At both, tropical and temperate sites, fine benthic organic matter (FBOM) was sampled using a hollow cylinder device placed in a flat area on the stream bed. Sediments in the bottom were vigorously agitated and 1L of the confined water was filtered (250 mL) using pre-weighed glass fiber filters (47 mm diameter GFF Whatman filters, USA) with a field vacuum pump. Three samples were collected at each sampling occasion. Each filter was transported to the laboratory into an aluminum envelope within an ice cooler and froze at -20° C until be oven dried (70° C for 48 h), burned (550° C for 4 h) and weighed to estimate ash free dry mass (AFDM), which was expressed in mass weight per area

(g/m^2) for each date. FBOM was averaged for the dates collected at each sites to perform further analyses (detailed below).

At both, tropical and temperate sites, coarse particulate organic matter (CPOM) was collected along three random transects perpendicular to the stream, using a surber net bottom sampler (30 cm x 30 cm; 0.5 mm pore size). The CPOM samples collected across each transect were transported in individual Ziploc bags to the laboratory in an ice chest. Once in the laboratory, samples were air dried for 24 hours, oven dried (70° C for 48 h), weighed and ignited (550° C for 4 h) to calculate the AFDM. Due to logistical constrains the CPOM samples in Colorado were not ignited, but their AFDM was calculated multiplying the DM coefficient of Colorado samples by a correction factor (0.77), which was obtained from the relationship between DM/AFDM in the Ecuadorian samples collected in this study and calculated with unpublished data collected in a decomposition experiment carried out in Portugal by several authors (Capps et al. 2011; Graça and Poquet 2014; Ferreira 2014, personal communications).

Litter decomposition experiment

Microbial litter decomposition was measured using the litter bag method (15 x 17 cm, 0.5 mm mesh size). *Alnus acuminata* Kunth (tropical species) leaves were gathered directly from trees in two locations ($0^0$14'46"S; 78^0 0'21"W and $0^0$22'0"S; 78^0 8'0"W) during April and May 2013. Leaves were dry stored until needed. Bags containing 5.00 g (± 0. 02 S.D.) of litter were deployed in the streams on August 29th of 2013 and six replicates were retrieved from each stream and at each sampling time after 3, 12, 30 and 60 days. *Alnus incana* (L.) Moench (temperate species) leaves were collected directly from several threes (40° 49'48"N; $105^0$43'32"W) during June 2013. Five replicates with portions of 4.00 g (± 0. 02 S.D) were deployed in each stream site on July 26th of 2013 and then recouped after 3, 12, 23 and 28 days. Experimental time in the temperate site was

shorter due to fast decomposition rates, even when the planned dates were the same as the tropical latitude.

Litterbags were placed in stream sections with moderate velocity and depth; they were laid on the bottom and tied to a rod. Samples were recovered according the scheduled dates in individual Ziploc bags and transported to the laboratory inside a chest with ice. In the laboratory they were gently washed with abundant distilled water to remove sediments. Leaf material was air dried for 24h and then oven dried (70° C for 48 h), weighed, ignited in a muffle (550° C for 4 h) and weighed again to calculate the ash free dry mass (AFDM). Three additional replicate bags were transported to the field and returned to the laboratory the first date of the experiment (Day 0) to calculate the loss mass by handling and to determine the corrections for air:oven dry mass (AFDM).

Comparison of litter decomposition between Alnus species

Since litter breakdown is affected by the chemical and physical quality of the leaves and by environment conditions, I carried out a second essay with leaves from both plant species in a same location (under identical environmental conditions) to investigate the importance of the environment and intrinsic leaf characteristics differences in decomposition rates. Fine mesh litter bags containing leaves of *Alnus acuminata* Kunth. and of *Alnus incana* (5 and 3 replicates of each) were incubated in the Ecuadorian site E2838. The leaves were deployed in 5 October and recovered in 7 November 2014. They were transported to the laboratory, dried and weighed as before to determine mass loss.

Initial litter quality

Initial specific leaf area (SLA; as a surrogate of toughness), expressed as $cm^2\ g^{-1}$ was determined in 10 leaf discs (10 mm) taken with a cork borer from different leaves. Leaf discs were oven dried, weighted and registered. Initial concentration of Nitrogen (N),

Carbon (C), Phosphorus (P), total phenols and lignin content was determined from grinded (Retsch, ZM100) and oven dried at 105° C, 24 h (total phenols, lignin and phosphorus) and at 60°C, 7 days (nitrogen and carbon). Polyphenolics were extracted from 100 mg of leaf powder (n=4) with 70% acetone and quantified by the Folin Ciocalteu method g (Bärlocher and Graça 2005). Lignin was extracted from 0.5 g (±0.1 mg) leaf powder portions (n=6) with 72% H_2SO_4 and quantified by the Klason lignin method (Goering and Van Soest 1970). Phosphorous was extracted from 0.002 (±0.1) mg leaf powder portions (n=4) with NaOH 1M and $Na_2S_2O_8$ 0.5M (American Public Health Association 1998). Carbon and nitrogen were measured from 0.5 (±0.2) mg leaf powder (n=4), were weighted and measured in an EA-IRMS (Thermo Delta V Advantage Conflo III and Flash EA 1112 series).

Considering that *Alnus acuminata* Kunth leaves (tropical species) were collected in two localities but did not show significant differences of remaining mass loss in the first experiment (p = 0.66; t-test), the leaf quality analyses were carried out with no distinction between sites.

Data analyses

Environmental parameters: In the tropical sites, stream physical and chemical parameters across the five sites were compared by analysis of variance (ANOVA) or Kruskal-Wallys (H) when the data was not normally distributed. Dissolved oxygen data was subjected to arc-sin transformation since original data was in percentage. Analyses were computed with SigmaStat 3.5 statistical software. ANOVA tests were not calculated for the temperate system since parameters were measured only once.

Litter decomposition: Decomposition rates (k/d) were computed as the slope of the regression of ash free dry mass remaining (log) over time. To account for temperature

variations across sites, decomposition rates were also normalized using degree days (*k*/dd), substituting in the previous model the time for accumulative mean daily temperature. In the case of the site E1805 (that did not have the temperature logger) the accumulated temperature during the experiment was determined assuming an average temperature from one date to the next one.

Relation between the environment and decomposition: To determine the importance of environmental factors on litter breakdown, I performed a linear regression between decay rate (k/d) and the environmental variables which were significant different in the ANOVA test with STATISTICA 7 software (StatSoft, OK, USA). Since dissolved oxygen and conductivity were autocorrelated with temperature in the tropical stream (Pearson and Spearman coefficients; SigmaStat 3.5 software), they were not considered as variable responses.

Temperature coefficient (Q_{10})

Temperature coefficient was computed for both latitudes using the following function:

$$Q_{10} = \left(\frac{k2}{k1}\right)^{\left(\frac{10}{T2-T1}\right)}$$

Where k_2 and k_1 are the observed decay rates constant for the decomposition process at two temperatures, T_2 and T_1. Values of Q_{10} >1 means that the rate of the reaction is completely dependent of the temperature (Kirschbaum 1995).

Toughness, nitrogen, phosphorous, polyphenolics and lignin content for both species of leaves, were compared by a pair unbalance t-test of two tails. T-test was also used to compare decomposition rates of *A. incana* in Ecuador and Colorado, and decomposition rates of *A. acuminata* in both experiments.

2.3 Results

Streams characterization

Stream water temperature in Ecuador ranged from 6.9 °C in the highest site to 17.4 °C in the lowest site, whereas in Colorado it ranged from 6.5 – 14.9 °C (Table 1). Ecuadorian streams' substrate was composed mainly by cobble and pebbles and densely covered by vegetation, except in the highest site where the boulder was the main substrate and the canopy cover was scarce. In Colorado substrate was dominated by sand and gravel and all sites were canopy covered (Table 2).

In Ecuador streams were moderate alkaline and oxygenated (Table 3). They were not different in terms of CPOM (p = 0.249; F = 1.476; df = 4) nor FPOM (p = 0.190; F=1.705; df = 4); the variability was high in some streams (Figure 1).

Only four parameters differed along the gradient: temperature, conductivity, dissolved oxygen and levels of phosphates and sulfates in the water (Table 4). Temperature, conductivity, sulfates and oxygen decreased, while phosphorous increased with elevation. Some of such relationships were linear (temperature p = 0.002; R^2 = 0.87; dissolved oxygen p = 0.001; R^2 = 0.99; phosphorous; p = 0.016; R^2 = 0.23). Additionally, conductivity and pH displayed a significant negative relationship with the altitude (p = 8E-06; R^2 = 0.68 and p = 0.013; R^2 = 0.25, respectively), as well as sulfates (p = 0.005; R^2 = 0.29). In Colorado the environmental data set was nor so complete, but temperature also decreased with altitude (regression analysis, p = 0.010; R^2 = 0.92) no tendency for altitudinal changes was observed (Table 5). None of the other parameters were linearly related with elevation (p > 0.05).

Nitrates in Colorado were one order of magnitude higher than in Ecuador (11.28 vs. 0.40 µg/L; balance t-test with one tail, p = 0.04), but levels of phosphates were not statistically different (6.75 µg/L in Colorado vs.11.29 µg/L Ecuador; t-test, p = 0.05), as in

discharge (0.017 in Colorado vs 0.14 m^3/s in Ecuador; t-test, $p = 0.05$).

Litter decomposition

In Ecuador, breakdown of *Alnus acuminata* leaves ranged from 0.0065 to 0.0144 k/d and decreased with increased altitude (linear regression, $p = 0.00064$; $R^2 = 0.96$). In Colorado, the breakdown rates of *Alnus incana* ranged from 0.0197 to 0.0453 k/d without any tendency along the altitudinal gradient (linear regression, $p = 0.48$; $R^2 = 0.0005$; Table 6; Figure 2).

When the decay rates (k/d) were expressed in terms of degree days (k/dd), differences of decomposition rates among the tropical streams and along elevation disappeared (linear regression, $p = 0.002$; $R^2 = 0.036$). The global decomposition rate in k/dd for the Ecuadorian was -4E-08. In Colorado the faster decomposition in degree days was observed in the three highest sites, compared with the two lowest sites (0.0016 – 0.0044 *k*/dd; $p = 0.76$; $R^2 = 0.402$; Figure 3).

Litter decomposition rates in Ecuador and Colorado were not related with phosphorous levels (linear regression; $p = 0.17$; $R^2 = 0.04$ and $p = 0.20$; $R^2 = 0.633$) and nether with nitrogen (linear regression; $p = 0.80$; $R^2 = 0.003$ and $p = 0.68$; $R^2 = 0.06$). The same applies when the results were expressed in degree days (linear regression; phosphates: $p = 0.08$; $R^2 = 0.13$ and $p = 0.57$; $R^2 = 0.19$; nitrates: $p = 0.63$; $R^2 = 0.01$ and $p = 0.20$; $R^2 = 0.47$).

Therefore only in the tropical streams litter breakdown were explained by temperature. In the temperate streams other environmental factors seems to be influencing strongly the decomposition

Finally, the Q_{10} in tropical and temperate streams were 1.89 and 1.27, respectively. Both factors calculated to determine the dependence of the decomposition rate with the temperature were above 1, which means that litter breakdown in both latitudes are

completely dependent of the temperature.

The analyses of these contradictory results will be discussed later on.

Comparison of litter decomposition between Alnus species

Litter decomposition was faster in Ecuador than in Colorado (k = 0.0098 vs. 0.0262; $p < 0.0001$ – t-test). To investigate whether differences were due to (a) litter quality, (b) environmental conditions or (c) latitude, I firstly analyzed the litter properties.

A. acuminata leaves had higher lignin content and low SLA compared with *A. incana* which was soften. Polyphenolics content in *A. acuminata* was three times higher than its counterpart. *A. incana* had lower nitrogen and high phosphorous than *A. acuminata* (Table 7). Therefore, except for nitrogen, *A. incana* was a better quality substrate for decomposers than *A. acuminata*.

When exposing leaves of both species under the same environmental conditions (site E2838, Ecuador), breakdown rates of the tropical *A. acuminata* were still slower than *A. incana* (unbalance t-test with two tails; $p = 0.038$), although its k values increased 61% compared with the first experiment. There were no differences in breakdown rates of *A. incana* leaves when exposed in both latitudes (unbalance t-test with two tails; $p = 0.11$; Figure 4).

2.4 Discussion

Since the latitude and the elevation can be used as a surrogate of the temperature (Rahbek 2004) and considering that metabolic activity lies in the thermodynamic laws (Brown et al. 2004), this study assessed the relevance of the temperature as the main environmental factor controlling the microbial decomposition in freshwater systems along an altitudinal gradient in the tropical and temperate latitude.

As hypothesized, for the tropical system, decomposition decreased with increased

altitudes. The results, were consistent with the study carried out along an altitudinal gradient in a temperate river by Taylor and Chauvet (2014), in which small rises of temperature increased the litter breakdown rates. This trend was confirmed when the temperature effect were factored out (degree days-1). However, contrary to my hypothesis, Colorado streams did not exhibited the expected tendency despite the water temperature changes with elevation. Considering that the positive trend was deployed only in the tropical system, other variables might be influencing strongly the breakdown rates. Such variables will be discussed further down.

According to the MTE, microbial metabolism is predicted to increase with temperature and consequently, accelerating the litter processing. Since the enzymatic activity depends directly on temperature to degrade the substrate (Peterson et al. 2004), fungal enzymes would catalyze faster the transformation of organic matter as the temperature increases. Laboratory experiments have determined that the magnitude of the stimulation on litter decomposition depends on the fungal optimum ranges of the aquatic hyphomycetes species involved (Fernandes et al. 2009, 2012; Geraldes et al. 2012), considering that sensitivity of the enzymatic activity is affected when the temperature rises or decreases out of their optimal limits (around 10° C; Ferreira et al. 2014) . Therefore, the higher decomposition rates recorded at lower altitudes in the Ecuadorian gradient, suggest that fungal communities at higher elevations might be still below of their thermal optimum limit, which is translated in lower metabolic activity of aquatic hyphomycetes that slowdowns the leaf litter processing.

The temperature dependence of the microbial leaf litter decomposition in Ecuador was corroborated with the thermal coefficient value ($Q_{10=}$ 1.89), which is comparable with other biological processes such as soil respiration ($Q_{10=}$ 2.4; Raich and Schlesinger 1992) and terrestrial decomposition ($Q_{10 =}$ 2.12; Kätterer et al. 1998).

Contrary to the tendency found between the decomposition rates and the

temperature along the altitudinal gradient in Ecuador, Colorado streams did not display any relationship with the temperature as the main variable controlling this process, despite the Q_{10} value (1.27) determined the opposite. Probably, there are other environmental factors such as nutrients in water (Ferreira et al. 2014), in this case specifically the high nitrogen concentration and microbial community assemblages (Fernandes et al. 2012) that are affecting the decomposition, overriding the temperature effect determined by the thermal coefficient.

Nitrogen and phosphorous have been identified as limiting macronutrients responsible to affect decomposition in lotic freshwater ecosystems (Suberkropp and Chauvet 1995; Gulis and Suberkropp 2004; Hobbie 2005; Ferreira et al. 2006). However, none of these nutrients were correlated with the litter breakdown. Studies of litter decomposition in terrestrial systems reveal that limiting concentrations of micronutrients might affect the litter breakdown rates (Hobbie 2008; Kaspari et al. 2008; Powers and Salute 2011). For instance, Manganese (Mn) constrains could reduce the lignolytic activity of microorganisms (Kaspari et al. 2008) and the lower levels of Postasium (K) could diminish the cytoplasmic transmission, restricting the substrate colonization and resource uptake (Powers and Salute 2011). Probably, the absence of the trend along the temperate gradient could be the result of a great variability of the local environmental variables, which should be investigated further.

In terms of latitudinal comparisons, lower decomposition rates were recorded in Ecuador than in Colorado, contrary to the expectations. Decay rates (k/d) values recorded in the current study for tropical and temperate streams were consistent with the literature (0.0060 and 0.017, respectively) using *Alnus* leaves as substrate (Gonçalves et al. 2006; Ferreira et al. 2012). Some studies have documented slower microbial litter breakdown rates in tropical streams compared with their counterparts in the temperate areas (Gonçalves et al. 2007; Boyero et al. 2011). Then, even when the microbial decomposition

depends of the temperature and should be faster as it increases toward to the tropics, the opposite pattern recorded in this study might be the result of certain regional factors that could override the temperature effect.

The incubation of both leaf species in the same stream site (E2838) in Ecuador reveled that differences in litter breakdown between Ecuador and Colorado were partially due to litter quality and partially due to environmental local conditions. Since the litter processing is affected by the leaf quality (Baldy et al. 1995; Ferreira et al. 2012; Schindler and Gessner 2009), researchers have employed the use of a single species (e.g. *Alnus glutinosa*) to compare the decay rate among different ecosystems (Gonçalves et al. 2007; Boyero et al. 2011). Nevertheless, the use of exogenous species may lead to misconstrue the results because the performance of the decomposers will not reflect what happens in natural conditions. In order to overcome this issue, the current research used native *Alnus* species.

The high lignin content and the elevated production of secondary metabolites (polyphenolics), as well as the poor nutrient quality (low C:N ratio) of the leaves in tropical areas, have been documented as an evolutionary strategy of plants against the high herbivory rates (Coley and Barone 1996). The higher concentrations of polyphenolics in *A. acuminate* might retard the microbial colonization. However, due to these secondary compounds are easily washed out in the leaching phase of the process (Graça and Cressa 2010), others intrinsic leaf factors seems to be more relevant. In lotic systems, the lignin content has been identified as the major factor limiting the decomposition (Hobbie 2005; Suberkropp and Chauvet 1995; Fernandes et al. 2012). Since the lignin-rich content in the leaf provides higher toughness (Perez et al. 2000), higher investment of microbial decomposers to degrade this constitutive compound is required (Gonçalves et al. 2007; Graça and Cressa 2010; Ferreira et al. 2012). Despite *A. acuminate* had higher nitrogen content compared with *A. incana*, the higher toughness in the tropical leaf might be

limiting the fungal activity and as result, the litter decomposition was slower in the tropical latitude. Lower phosphorous levels in the tropical leaf would be an unlikely explanation limiting the decomposition because it is quickly washed in the initial stage of the leaching phase (Sangkyu and Kang-Hyun 2003; Gessner et al. 2010).

The higher loss mass exhibited by *A. acuminata* leaf in this second experiment is beyond of my scope, nonetheless it might be due to higher nutrients in the water (data no recorded) and the increment of 2.3° C of temperature that correspond to 52.4 degree days of difference (data not shown) between the experiments, which may lead to hasten the process. The synergistic effect of both variables might enhance the microbial activity and as a result, the decomposition process was faster (Ferreira and Chauvet 2011a) . On the other hand, the lower decomposition rate of *A. incana* might be related with the fungal assemblage community in the site, which probably was not efficient enough to degrade this foreign substrate, as have been suggested by Gonçalves and collaboratores (2007).

Considering the higher energy cost that represents to uptake nutrients directly from the substrate (Ferreira, et al. 2014), microbial decomposers may be using preferentially nitrates from the water to compensate the low nitrogen content of *A. incana* leaf. The velocity of the enzymatic reaction can be measured based on the substrate availability by the Michaelis-Mentel model and according to the results published by Ferreira and collaborators (2006), the litter breakdown rate (degree days^{-1}) increases exponentially when the concentrations of nitrates in water range between 0 and 100 µg/L. The higher levels of nitrates registered in Colorado streams suggest that even when decomposition is dependent of the temperature (Raich and Schlesinger 1992; Tjoelker et al. 2001), nitrates concentrations in water hasten the process, as reported for other authors (Gonçalves et al. 2006, 2007). Because in the tropical sites the nitrates levels in water were significantly lower (one order of magnitude difference), the temperature effect in the decomposition rates was clearly detected.

In summary, the results obtained in this study suggest that microbial decomposition is ruled by the temperature, as predicts the MTE. Nevertheless, leaf physical and chemical features such as the poor leaf quality (high lignin content) (Hobbie 2005; Fernandes et al. 2012) on the tropical leaves, delayed the litter breakdown. Concurrently, in the temperate system the microbial decomposition was hasten by the higher nitrogen concentrations dissolved in water. It seems that even when the microbial decomposition is temperature dependent, this process is strongly influenced by inherent local factors that can nullify the temperature effect along the altitudinal gradients. Considering that decomposition includes microbial and macroinvertebrates decomposers, future experiments are required to determine if the overall process would exhibit the same temperature dependence obtained in this study.

3. CHAPTER II

Microbial decomposers diversity in tropical and temperate streams along an altitudinal gradient

3.1 Introduction

The latitudinal increment in species richness towards the equator, has been recorded for several organisms in terrestrial, marine and freshwater environments (Gaston 2000; Hawkins 2001; Hawkins and Felizola 2004). Nevertheless, this unimodal trend differs among taxonomic groups (Willig et al. 2003) and have not been well stablished for microorganisms such as fungi and bacteria, probably because their lack of knowledge, high abundance and cosmopolitan distribution (Fierer et al. 2007, 2011).

Different hypotheses have been proposed to explain the latitudinal variation in species richness. Those include the larger areas of tropical systems, primary production gradients, climate patterns and stability, biotic interactions such as the intensity of predation and competition, as well as the evolutionary history of the species (Krebs 2001; Molles 2010). If high number of taxonomical groups follow this ubiquitous tendency, there should be a prevailing component along the different geographical habitats shaping this patterns of species richness (Hawkins 2001; Martiny et al. 2006). Then, since temperature changes gradually from the poles towards to the tropics, as happens with the species richness, the evolutionary adaptations of the organisms and their habitat colonization might be explained broadly by this environmental factor and others that covariate with it (Rosenzwig 1992; Gaston 2000).

Higher latitudes exhibit larger annually temperature fluctuations compared with the tropics, where only daily seasonality is documented (Janzen 1967; Sarmiento 1986; Ghalambor et al. 2006). Consequently, due to evolutionary adaptations, tropical organisms living at lower latitude should experience narrow thermal tolerance ranges that constrain their habitat niche and lead to harbor higher species diversity (Janzen 1967). Additionally, since the temperature also decreased with elevation, is reasonable to expect that the altitudinal gradients mirror the latitudinal species richness changes. High altitude

sites can be considered as isles from the biogeographic point of view (MacArthur and Wilson 1963), because their smaller area (Lomilino 2001) and their distance between mountain tops, lower number of species is likely to inhabit high elevation sites.

The spatial distribution patterns of the local species richness (α diversity) and within a region (γ diversity) has been widely documented (Rosenzweig and Sandlin 1997). However, less attention has been given to the variation of species along habitat gradients such as the elevation. The degree of species changes across habitats and along gradients is known as β diversity (Whittaker 1972). Since species are evolutionary adapted to occupy a certain niche constrained by their physiological adaptations (Janzen 1967), along an habitat gradient the species composition is likely to experience changes because they might display niches overlap.

The rate at which the air temperature changes in terrestrial systems are more extreme than in the aquatic environments (Molles 2010), due to the thermal capacity of water (Doods 2002). As a consequence, aquatic species are likely to exhibit different patterns of adaptations in response of these temperature variations. Hence, the thermal tolerance limits in the tropical organisms might be expected to be wider, but there is still expected to find differences between the tropical and temperate latitudes if the temperature is leading the species distribution.

In low order streams, the input of allochthonous organic matter is consider one of the main energy source for aquatic biota, which include aquatic fungi and bacteria. Aquatic hyphomycetes, accounts more than the 87% of the total microbial biomass associated to the decomposing leaves in forested streams in temperate systems (Baldy et al. 1995), for that reason, and because they produce enzymes capable of decomposing leaves, they are considered to be the main decomposers in stream (Hieber and Gessner 2002; Baldy et al. 1995), increasing the nutrient content and facilitating macroinvertebrates feeding through softening the leaves (Graça 2001). However, bacteria

main gain more relevance at later stages of leaf litter decomposition (Baldy et al. 1995; Gessner and Chauvet 1997). Hence, fungi could be important decomposers of CPOM while bacteria in FPOM (Gessner and Chauvet 1997). Therefore, both would have complementary roles in the lotic decomposition (Graça 2001).

Aquatic hyphomycetes and bacteria may exhibit synergistic relationships if their enzymatic capabilities are complementary (Bengtsson 1992; Gulis and Suberkropp 2003). Nonetheless, it was also postulated that fungi and bacteria may interact antagonistically (Gulis and Suberkropp 2003) and compete for the limited amount of nutrients in leaf substrate (Schneider et al. 2010).

The potential dispersion and the high density described by microorganisms, may explain the ubiquitous distribution of unicellular organisms such as bacteria. In marine environments, the richness of planktonic bacteria displays a strong positive correlation with temperature as the latitude decreases (Fuhrman et al. 2008). In lentic freshwater systems, the distribution of bacteria is mainly controlled by pH and temperature (Lindström et al. 2005). The positive correlation between the bacteria distribution with the pH has also been described for terrestrial bacterial communities, which tend to decreases as the soils becomes more acid independently of the altitude, the latitude and temperature (Lindström 2000; Fierer and Jackson 2006; Fuhrman 2009). Bacterial decomposers vary greatly according the substrate characteristics and contrary to fungi, the richness of bacteria taxa tend to increase toward the end of the decomposition (Das et al. 2007). Studies along an altitudinal gradient and between different latitudes with terrestrial bacteria colonizing the surface soil and leaves demonstrated the absence of the unimodal pattern described for larger organisms (Fierer and Jackson 2006). However, there is still a barely knowledge regarding how the species richness and composition of bacteria involved in the litter breakdown in streams vary along temperature gradients in a broad spatial scale.

Regarding aquatic hyphomycetes, they exhibited a cosmopolitan distribution, although some taxa may have a more restricted distribution (Wong et al. 1998). In the only study across several latitudes, aquatic hyphomycetes were shown to be more diverse at middle latitudes, around the temperate and Mediterranean areas (Jabiol et al. 2013). Nevertheless, the lack of studies comparing the fungal species richness along an altitudinal gradient and nether the thermal tolerance limits of taxa at different latitudes.

Traditionally, the identification of aquatic hyphomycetes was based on the conidia morphological characteristics (asexual spores), which is highly time consuming and depends on the capability to obtain conidia under laboratory conditions (Duarte et al. 2007; Bärlocher and Marvanová 2010). Fingerprinting techniques allow the identification of genetic variation within a community and for this reason, the use of molecular techniques assessing the polymorphisms of the ribosomal genes, located within the RNA sequence (rRNA), have been implemented as a useful tool for phylogentic and taxonomical analyses (White et al. 1990, Belliveau and Bärlocher 2005; Bärlocher 2010). Nuclear Internal Transcribed Spacer (ITS_2 region) is a suitable marker to assess the aquatic hyphomycetes taxa diversity (Belliveau and Bärlocher 2005; Duarte et al. 2007, 2010). For bacteria the marker is the 16S rRNA sequence within the small ribosomal subunit (Muyzer et al. 1993; Karp 2005). In microorganisms the use of fingerprinting techniques, such as the denaturing gradient gel electrophoresis (DGGE) allow to visualize bands, which are considered as operational taxonomic unit (OTU), which are not equivalent to species in large organisms (Fuhrman 2009).

Here I test several hypotheses regarding the biodiversity changes along altitudinal gradients in two latitudes, using molecular biology techniques to assess decomposers diversity. Since prevailing temperatures may determine the thermal tolerance ranges of the organisms as was proposed by Janzen, the temperature is likely to affect the number of microbial species and the community composition, which can be translated in the

modification of the leaf litter decomposition. Laboratory experiments have determined that the identity of species involved in this process, matters more than the species number (Fernandes et al. 2012; Geraldes et al. 2012; Duarte et al. 2013). Considering that higher latitudes exhibit higher seasonality during the year compared with the tropical systems, where the organisms' dispersion is constrained by their thermal tolerance ranges, the species richness of microbial decomposers (aquatic hyphomycetes and bacteria) is expected to be higher in the tropical latitude than in the temperate regions, according the Janzen hypothesis (Janzen 1967). In the same way, due to the temperature variation as the elevation increases, the number of microbial taxa (fungi and bacteria) would decrease with the altitude, as has also predicted by the Biogeography Island theory, because the area is larger in the lowlands. Furthermore, due to the physiological constrains of the organisms, the species turnover in the tropics is likely to be higher than in the temperate areas, where higher similarity of taxonomical groups are expected to be shared within the gradient.

To test these hypotheses I assessed the taxa richness and community composition of microbial decomposers (aquatic fungi and bacteria) involved in the leaf litter processing in10 low order streams along an altitudinal gradient in a tropical (Ecuador) and temperate latitude (Colorado, USA; see previous chapter) by the DGGE technique. Leaves of *Alnus acuminatada* and *Alunes incana* were deployed in fine mesh bags (0.5 mm) in 5 sites along altitudinal gradients in Ecuador (1645-3847 m.a.s.l.) and Colorado, USA (1992-3200 m.a.s.l.), respectively. The methodology is detailed in chapter 1.

3.2 Methodology

General information

Leaves of two riparian trees in Ecuador and Colorado were used in litter breakdown experiments. Portions of leaves were enclosed in fine mesh bags and incubated in streams along altitudinal gradients in both systems to measure decomposition and associated microbial communities. The experimental design is detailed in chapter 1.

Microbial DNA samples

Primary material for DNA extraction was obtained from three replicate of fine mesh bags (0.5 mm mesh size; 5 x 5 cm) containing 2 to 3 alder leaves: *Alnus acuminata* in Ecuador and *Alnus incana* in Colorado (Table 1, chapter 1). Replicate bags were recovered after 3, 12 and 32 days in tropical and 3, 12, 23 and 28 days in temperate streams. Samples were washed with distilled water, placed into an aluminum envelope, transported in liquid nitrogen to the laboratory and storage at -80° C until being processed.

Microbial DNA extraction and amplification

Fungal and bacteria DNA extraction was performed with MoBio Power Soil DNA Isolation Kit™ using 3 freeze-dried leaf discs of 12 mm of diameter (¼ of 4 leaf discs was cut from each replicate and pooled) following the manufacturer's instruction (Annex 5). DNA was eluted in 100 µl of 10mM Tris buffer, storage at -20°C and used as template for PCR amplifications. Replicates bags were pooled and treated as one sample to maximize the number of taxonomical units by site.

Microbial diversity of the 35 samples was assessed in two independent amplification reactions with different primer sets. To assess the fungal assemblage the ITS2 region of the rDNA sequence was amplified using ITS3GC/ITS4 pair primers. Bacteria were analyzed with the primer set 338GC/518 that targets the V3 region located in the 16S rDNA sequence. The total volume of the amplification reaction was performed in 25 µl containing 1X of GoTaq® Green Master Mix (Promega), 0.4 µM of each primer set and 1 µL of template DNA. Polymerase chain reactions were performed in a T100™ thermocycler (BIO-RAD) following the protocol detailed by Sahadevan et al. (2012) with an initial denaturation at 95°C for 2 minutes; 36 cycles with a denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and elongation at 72°C for 1 minute; and a

final polymerization at 72°C for 30 seconds. To confirm the amplification of the target sequences with both primer sets, PCR products were resolved by electrophoresis in a 2% agarose gel at 80 V for 30 minutes with an expected size product between 380 and 400 bp for fungi and 200 bp for bacteria.

Denaturing gradient gel electrophoresis (DGGE)

PCR confirmed products (20 µl) were resolved in an 8% polyacrylamide denaturing gel in 1X TAE (Tris Acetate-EDTA) and run at 56°C, 55 V during 16 hours in a DCode™ Universal mutation Detection System (BIO-RAD). Three acrylamide gels were prepared for fungi and bacteria, separately. The denaturing gradient used for fungal amplification products was 40% to 70% and for bacteria 30% to 70%, where 100% denaturant corresponds to 40% formamide and 7M urea (Annex 6). For gels alignment purposes, the extremes lines of each gel were occupied by 20 µl of a DNA mixture containing 4µl of 9 aquatic hyphomycetes species and 4 bacteria species, pooled separately according the electrophoresis run. The species used for the DNA mixtures were extracted and amplified with the same protocol described above from isolated strands which are deposited in the Biodiversity Laboratory at the University of Braga (Annex 7).

To visualize the fingerprinting pattern on the polyacrylamide matrix, the gel was stained with 10 µl of Midora Green® and incubated during 10 minutes at 18°C in darkness and agitation at 40 RPM. The images were captured under UV light in a transiluminator (Molecular imager® Gel Doc™ XR, BIO-RAD) using the Quantity One XMS software (BIO-RAD).

Statistical analyses

For the microbial analyses, the alignment and normalization of the acrylamide gels were performed with the BioNumerics software (version 5.0). To align the three gels of

fungi and bacteria, the bands selected in the reference line were the ones strongly marked and clearly separated from each other. Once that the bands were selected in the first gel, the same bands were chosen in the reference lines from the remaining gels, in order to make the comparisons possible among them. Band selection in the samples lines were accomplished with the following criteria: 1) clearly and defined bands with black and white background; 2) ambiguous bands were marked if they showed a define peak comparable with the bands previously selected. Smearing was assumed when the profile of the peak was bolder and excluded from the analyses. The software generates a matrix based on the height and intensity of the bands, which was used for further analyses (BioNumerics software 5.0 Manual).

Each DGGE band selected was considered as an operational taxonomic unit (OTU), taking into account that more than one species could migrate to the same position in the gel. The number of OTUs for each stream was recorded by time counting the bands in the respectively line, whereas the total number of OTUs by site was determined considering that DGGE bands that appear at the same height for all sampling dates must be counted only once. Fungal and bacteria assemblages were analyzed independently.

To determine if the time or the altitude influences on the number of microbial taxonomic units (fungi and bacteria, independently), an Analysis of Variance (ANOVA) was performed for Ecuador and Colorado. The homocedasticity assumption of the data was corroborated with the Barlett test, as well as the normality of the model applying the Shapiro test (R studio). The relationship between total number of OTUs at stream and the elevation was investigated with a regression analysis (OTUs vs. elevation). To identify the causes of OTUs along the altitudinal gradient in Ecuadorian and Colorado, environmental parameters were normalized and related using the Principal Components Analysis (PCA) with Primer v6. Afterwards, the five first axes scores obtained from the PCA were correlated with the total number of OTUs for fungi and bacteria, separately. For Ecuador

the conductivity and oxygen were excluded of the analyses because were highly correlated with temperature. In the case of Colorado, the PCA was performed excluding phosphates because of missing data in site C1992.

In a next step and to investigate taxa turnover along the elevation gradient, only three comparable altitudes were selected (3415, 2838 and 1805 m in Ecuador and 3200, 2798 and 1992 m in Colorado) to allow further comparisons between latitudes. The similarity among samples was calculated with the Bray Curtis index followed by ANOSIM analyses, in which values near to 1 means higher separation among factors, allowing the identification of taxa composition of microbial decomposers at each altitudinal gradient (indirect measure of taxa turnover).

Since fungi from terrestrial origin may remain in litter substrates during early stages of decomposition (Ferreira et al. 2012), day 3 was not included in data analyses. This assumption was confirmed in the exploratory analyses, in which samples from day 3 tended to form groups (cluster analysis). For consistency, the same was applied to bacteria. Two samples, one from Colorado (2590m, day 23) and one from Ecuador (1645m, day 32), were excluded from the fungal and bacteria analyses respectively, due to the methodological drawbacks in the PCR.

The total number of OTUs in Ecuador and Colorado were compared by a pair t-test. To identify the differences of species composition between latitudes, the output matrix from the BioNumerics software was exported, square root transformed and normalized using Bray-Curtis similarity to perform a metric multidimensional scaling (MDS) with 999 permutations and to build cluster applying the Unweightened Paire-Group Method Average (UPGMA) in Primer v6 software package. The differences among groups were calculated by the analyses of similarities (ANOSIM) using the same statistical software.

3.3 Results

3.3.1 Aquatic fungi

Altitudinal gradients

Ecuador

The number of fungal OTUs were not significantly different across sampling dates and altitudes (one way ANOVA; $p = 0.54$; F = 0.68; $df = 2$ for time and $p = 0.11$; F = 2.7; $df = 4$ by altitude; Figure 5.a). However, when the total number of OTUs is considered for each stream (all samples pooled), the number of aquatic fungi increased with the elevation ($p = 0.03$; $R^2 = 0.83$; Figure 6.a).

The two first axis of PCA explained 74% of the variability (three axis = 93%; Figure 7; Annex 9). The number of fungal OTUs was inversely correlated with the first PCA axis (r = -0.97; Annex 10). FPOM and PO_4 decreased along this axis. These results suggest that the main environmental factor influencing the OTUs numbers along the altitudinal gradient in Ecuador were phosphates in water.

Fungal composition on the three selected altitudes (3415, 2838 and 1805 m) along the tropical gradient were similar (ANOSIM; $p = 0.5$; $R^2 = 0.004$), meaning that streams share many taxonomical units of aquatic fungi among the analyzed streams.

Colorado

There were not significantly differences in the number of OTUs for the aquatic fungi when time and altitude were analyzed independently in Colorado (ANOVA; $p = 0.71$; F = 0.47; $df = 3$ for time and $p = 0.16$; F = 1.97; $df = 4$ by altitude; Figure 5.b). Unlike of the unimodal tendency recorded in the tropical gradient between elevation and the overall OTUs number by stream, the fungal richness in the temperate sites displayed a Gaussian curve with the elevation ($p = 0.7$; $R^2 = 0.57$), in which the maximum number of taxa was attained at 2212 m and then decreasing above and below this value (Figure 6.b).

The two main axis of the PCA for Colorado streams explained 82% of the variability (three axis = 92%; Figure 8, Annex 11). The number of fungal OTUs was strongly correlated with the third axis (correlation; r = -0.88), which, in turn, was correlated positively with NO_3 (0.629) and negatively with CPOM standing stock (-0.545; Annex 11). These results suggested that moderate concentrations of nitrate in water, higher amounts of CPOM and shallow waters could be responsible for the higher number of taxonomical units of aquatic fungi in the temperate studied system.

Species composition of aquatic fungi along the elevation zones (3200, 2798 and 1992 m) were different (ANOSIM; p = 0.003; global R= 0.5), particularly the highest and the lowest sites (p = 0.03; global R= 0.57 between 3200-1992 and p = 0.03; global R = 0.57 between 2798 and 1992).

Latitudinal comparisons

The number of fungal taxonomic units (OTUs) recorded in Ecuador (n = 13.3) and Colorado (n = 15.2) was not statistically different (t-test; p = 0.2; t =1.43; df = 8) between latitudes. The community composition of aquatic fungi differed between the tropical and the temperate latitude (ANOSIM*; p* = 0.001; global R = 0.69; Figure 9; see also Annex 13).

3.3.2 Bacteria

Altitudinal gradients

Ecuador

The number of bacteria taxonomical units did not significantly differ across time nor altitude in the five tropical streams (ANOVA; p = 0.57; F = 0.61; df = 2 for time and p = 0.08; F= 3.2; df = 4 by altitude; Figure 10.a). However, when the total number of OTUs recorded in the three sampling dates were pooled, lower altitudes in the gradient harbored

higher number of bacterial taxa (p = 0.0001; R^2 = 0.58; Figure 11.a).

The number of bacterial OTUs primarily correlated with the 2nd PCA axis (0.64), which in turn was correlated with CPOM standing stock (0.46), NO_3 (0.55) and NO_2 (0.53). Therefore lower number of bacterial OTUS tended to occur in sites with high concentrations of nitrates and nitrites, as well as with larger amounts of CPOM and lower concentrations of phosphates dissolved in water (Figure 7; Annex 9). ANOSIM analysis segregated bacterial communities among the three altitudes in the gradient (p = 0.54; global R = -0.021).

Colorado

Bacterial species richness (in terms of OTUs) did not change significantly with time and elevation (ANOVA; p = 0.13; F = 2.3; df = 3 by time and p = 0.12; F = 2.3; df = 4 by altitude; Figure 10. b). However, when OTUs were pooled by elevation, it was clear a strong decline with altitude (linear regression; p = 0.0004; R^2 = 0.99; Figure 11.b).

The variability of bacteria species richness in Colorado was strong and negatively correlated with the first PCA axis (r = -0.95; Annex 11). PCA analysis associated lower sites of the gradient (C1992 and C2212; high OTUs number), with high temperature and low discharge. Additionally, those sites were positive related with higher concentrations of nitrates in water and CPOM. Low OTUs, number were associated with deeper streams and higher current velocity (Figure 8; Annex 12).

Community identity changed significantly among the three selected streams (p = 0.003; global R = 0.51). Site C2798 was similar of C3200 and C1992 (ANOSIM; p = 0.11; global R = 0.27 between 3200 and 2798 and p = 0.06; global R = 0.41 between 2798 and 1992), whereas the extreme sites of the gradient were statistically different (p = 0.03; global R= 0.76).

Latitudinal comparisons

When Ecuador and Colorado samples are jointly analyzed, the number of bacterial taxonomical units (33 vs 32) were not significantly different (t test; $p = 0.84$; t = 0.21 $df = 8$). MDS analysis (stress value: 0.19) depicted no significant difference between latitudes (ANOSIM; $p = 0.41$; global R= 0.008; Figure 9 and Annex 15). It means that both systems harbor similar bacterial species in the both studied systems, the tropical and the temperate latitude.

3.4 Discussion

3.4.1 Aquatic fungi

Altitudinal gradients

Here I predicted a decrease in taxonomical units with increased elevation. However, this was not the case for aquatic fungi in Ecuador, where the number of OTUs increased with altitude, whereas in Colorado higher number of OTUs peaked in intermediate sites.

It is plausible that, besides (a) the increased daily temperature variation with altitude and (b) the island effect, other factors could be more important determining diversity. The evidences from this study are that nutrients could be such factors. Microcosms experiments have shown that high levels of nutrients (nitrates and phosphates) allow the coexistence of a larger number of aquatic fungi species (Grattan and Suberkropp 2001; Bärlocher and Corkum 2003). In Ecuador, phosphorous in water increased with elevation, and so the number of fungal taxonomical units, suggesting this nutrient could be controlling for fungal diversity. The importance of phosphorus to fungal growth was previously demonstrated in foregoing studies (e.g. Suberkropp and Chauvet 1995; Kavahagh 2005).

The same could explain changes in OTUs numbers in Colorado, although with lesser intensity. Moderate concentrations of nitrates and high quantities of CPOM standing stock were associated with the increment of fungal OTUs number. The outlier of this relationship is the lowest site (C1992) which exhibited the highest nitrate concentrations (50.9 µg/L) and the lowest number of taxonomical units (7). Some studies have documented that fungal diversity tend to increases at moderate eutotrophic conditions (Pascoal and Cássio 2004; Sridhar et al. 2005; Ferreira et al. 2014). I do not know why the site with highest nitrogen was the one with lower OTUs number. Potential explanations could be limitation by other factors (e.g. P, not measured), or an unmeasured perturbation.

The role of aquatic fungi as main decomposers of particulate organic matter in temperate streams has been widely recognized, as well as their association to the CPOM (Gulis and Suberkropp 2003; Krauss et al. 2011). The successful strategy to colonize a variety of substrate consists in the ability to get attach to the detritus by their conidia shapes and penetrate the vegetative tissues with the hyphae to uptake nutrients during the growth stage of their life cycle (Wurzbacher et al. 1998). The strong correlation of higher fungal taxonomic units to higher quantities of CPOM standing stock found in Colorado streams, suggests that nutrients may interact with substrate availability to explain fungal taxonomic richness.

Whatever the explanations for the OTUs distribution along the altitudinal gradients, it is clear that, although temperature daily variation and biogeography could be important, other factors are more prominent.

Community composition along altitudinal gradients

Assuming that the thermal tolerance range of the organisms would control the taxa distribution along the altitudinal gradient, I was expecting to find higher fungal taxa turnover in Ecuador than in Colorado, because the organisms would tend to occupy

narrow niches as a consequence of the temperature stability in the tropical regions. In Ecuador, taxonomical dissimilarities (as a measurement of β diversity) across sites were lower (i.e. higher similarity) than in Colorado, contrary to my expectations. The two highest Colorado streams were very similar, whereas the lowest site harbored different fungal taxa.

The most parsimonious explanation may be the geographic distance between the highest and lowest sites in the gradients (shorter in Colorado). There should be also consider that the Janzen proposal may not apply to aquatic fungi. Two reasons can be invoked. Firstly, the thermal range in the water is lower than on land, with minimum temperature never below 0º C and the maxima most likely never exceeding the 25º C (in the studied area). Hence, temperature limitation is less important for stream organisms. Secondly, microorganisms may have a larger thermal tolerance than large size organisms. If this is the case, then we should not expect differences in β diversity between tempered and tropical streams.

Latitudinal comparisons in taxa numbers

If the temperature is the main environmental factor leading the variability of species richness between latitudes, higher number of fungal taxonomic units will be expected in the tropical region, due to the narrow thermal tolerance of the organisms. However, Ecuador and Colorado had similar OTUs numbers. It has been proposed that highest species diversity of freshwater fungi is localized in temperate regions, at middle latitudes between 40-50° (Shearer et al. 2006). It is possible that fungal decomposers diversity do not follow the pervasive species richness gradient reported for the majority of the eukaryotic species (Willig 2003), as has been reported in the meta-analysis conducted by Hillebrand (2004), in which this pattern becomes weaker as the organism size decreases, an explanation fitting as well in the results discussed above.

The currently results should be interpreted with caution, considering that diversity in Ecuador could be underestimated due to the earlier stage of decomposition compared with Colorado, where the process was faster. For instance, in Ecuador by day 30 leaves already lost 30% of their mass, whereas in Colorado they lost 50% of their mass. It is plausible that in a comparable stage of decomposition, leaves in Ecuador could harbor a larger number of OTUs than the observed. Higher number of aquatic fungi recovered from submerged wood and identified by morphological techniques, was documented by Ho and collaborators (2001) in tropical and subtropical streams than in temperate sites for the north and south hemisphere, suggesting that longer tracking time in the process is required for future studies.

There were no differences in the number of taxa between Colorado and Ecuador, but the overall fungal community analysis (Cluster) segregated Ecuador and Colorado samples in two clades. This suggest that the two areas harbor unique fungal taxa, as has been reported on the literature for subtropical and temperate region (Ho et al. 2001). Since this work assessed the ITS2 region that is common for fungi, the use of molecular techniques allowed to identify that the fungal community composition in Ecuador an Colorado were different, but further studies should be performed in order to determine the different taxa identity focused in aquatic hyphomycetes. Given the methodology used, I was unable to identify the species, but in a side experiment carried out in Ecuador, in which fragments of leaves recovered from the five streams studied in this work were incubated to obtain spores and then isolated, I observed the presence of *Lunulospora curvula* (E2839), *Clavatospora longibrachiata*, (E3415), *Tetrachaetum elegans*, *Tetracladium marchalianum*, *Heliscus lugdunensis* and *Lemoniera spp*. (E1805 and E1645; Annex 16). The occurrence of these aquatic hyphomycetes species have been reported for temperate streams (Duarte et al. 2006; Garnett et al. 2000), corroborating that tropical streams share some aquatic hyphomycetes with temperate latitudes. However,

tropical systems may greatly differ from each other. For instance, Ferreira et al. (2012) record no colonization of litter by aquatic hyphomycetes.

Differences between Ecuador and Colorado in terms of fungi, could also been related with the litter substrate. Despite the nitrogen content was higher in *A. acuminata* (from Ecuador) than in *A. incana* (from Colorado; Table 7 chapter 1), which encourages a faster colonization of aquatic fungi (Fellman et al. 2013), the toughness and lignin were higher in the Ecuadorian species. Tough and lignin rich leaves are low quality substrate for fungi (Ferreira and Chauvet 2011a). Because of structural compounds (Andrew and Hughes 2005) and polyphenolics tropical litter has been classified as poor quality (Ferreira et al. 2012), which can constrain the number of fungal taxa in the tropics (Jabiol et al. 2013) as well as it may be influencing the fungal taxa identity (Bärlocher 2005).

Another factor that could explain fungal differences between Ecuador and Colorado was water nutrient content. Nutrients were shown to be important controllers of fungal taxa in previous studies (Ferreira and Chauvet 2011b). Nutrient concentrations differences observed in Ecuador and Colorado (0.4 µg/mL vs. 18.02 µg/mL; Table 5 chapter 1), may influence taxa numbers and taxa identity in those sites.

3.4.2 Bacteria

Altitudinal gradients

The number of bacteria taxonomical units decreased along the altitudinal gradient in Ecuador and Colorado, as hypothesized. This result conforms with the predictions of the Island Theory (MacArthur and Wilson 1963) and the temperature effect. Indeed, PCA analysis in both areas related OTUs richness with bacterial OTUs numbers (higher at lower sites). Nevertheless, it is plausible that temperature was not the pivotal environmental variable leading this biological variability. Higher quantities of CPOM standing stock and higher nitrates concentrations in water also influenced the diversity of

bacteria in lower sites at both gradients.

Studies have demonstrated that higher bacteria abundance and metabolic activity are positive correlated with larger surface areas of sediments and organic debris (Hargrave 1972; Yamamoto and Lopez 1985). Since heterotrophic bacteria require substrates with organic compounds as source of energy (Molles 2010) and higher quantities of coarse organic matter in tropical and temperate streams were associated with higher number of bacteria taxa, is likely to infer that more availability of organic substrate in the sites enhanced the probability to colonize organic substrates such as leaves, resulting in higher number of bacteria OTUs.

In both, Ecuador and Colorado, PCA suggest a relationship between nitrogen and OTUs numbers. Nutrients may allow high production and therefore high numbers and high diversity. However, most likely ecosystems are co-limited by several factors. For instance, Farjalla et al. (2002) observed that bacteria production is not stimulated by the addition of nitrogen in Amazonian freshwater ecosystems.

Additionally, the high number of OTUs recorded in the lower Ecuadorian site (E1645) might be interpreted with caution, because the assessed V3 region within the bacterial 16 rDNA sequence is common for any bacteria taxa (Case et al. 2007), meaning that the identification of OTUs is not selectively only for the bacteria associated with the litter decomposition, which may overestimated the number of taxonomical units in the site. Furthermore, higher number of fecal coliforms are apparently natural abundant in tropical systems (Boulton et al. 1998) and since the mentioned stream was near a farm, OTUs numbers may be responding to animal waste, which is consistent with higher concentrations of nitrites at this site (Table 3 Chapter 1).

In summary, although different environmental variables can be controlling OTUs numbers along the gradients in Ecuador and Colorado, results suggest that temperature may be a prominent factor, followed by CPOM standing stock, and nitrogen.

Community composition along altitudinal gradients

In Ecuador, OTUs similarity along the altitudinal gradient was high (*i.e.* low β diversity) whereas in Colorado the similarity was lower across sites, particularly the sites located at 2798 m and 3200 m, with lower similarity with the lowest site (1992 m). Therefore, β diversity in Colorado was higher than in Ecuador.

These results lesser the importance of temperature as a main environmental factor controlling bacterial species diversity, as expected if local organisms would be adapted to occupy narrow (Ecuador) or wide (Colorado) niches as a consequence of their thermal tolerance ranges (Janzen 1967).

These results were consistent with the observed for fungi and the explanations could be the same: (1) low thermal range of aquatic systems, (b) wide thermal tolerance for microorganisms and (3) other factors may override temperature effects. Probably the moderate levels of nitrates recorded in the stream C2798 offer more suitable conditions for different taxa which are restricted only in the highest and the lowest sites, constrained by the contrasting levels of nitrates (5.8 vs 50.9 µg/mL).

Latitudinal comparisons

The latitudinal gradient in species richness have been corroborated for larger and small organisms, such as planktonic marine bacteria and zooplankton (Fuhrman et al. 2008). Notwithstanding, this study recorded similar quantities of freshwater bacteria taxa in the tropical and the temperate latitude, which is consistent with other studies indicating null or weak latitudinal pattern (Hillebrand and Azovsky 2001; Hillebrand 2004; Fierer et al. 2007, 2011). The similarity on the taxa identity between Ecuador and Colorado streams stablished in this study, suggest that freshwater bacteria had a cosmopolitan distribution,

as has been documented in the literature for small size organisms. The most probable explanation for the absence of differences between latitudes in terms of OTUs number and taxa identity, might be due to the small organisms usually exhibit higher dispersal ability and may be less influenced by broader scale variables that regulates larger individuals (Hillebrand and Azovsky 2001; Hillebrand 2004; Fierer et al. 2007, 2011), making them capable to colonize broader areas and as consequence, diminishing spatial differentiation worldwide (Fuhrman et al. 2008).

Conclusive remarks

The present work reveals that biogeography of the aquatic fungi is highly influenced by the substrate type available (*Alnus* species) and by local factors such as nutrients dissolved in water (particularly phosphates in Ecuador). Because organisms at higher latitudes, experienced wider variability of temperature during the year and increasing their thermal tolerance ranges (Janzen 1967; Ghalambor et al. 2006), the species richness of fungi in the tropics was expected to be higher than the temperate region. However, the same number of taxa richness in terms of OTUs was recorded for Ecuador and Colorado. Given the temperature decreased with the elevation, the altitudinal gradient was expected to mirror the latitudinal gradient pattern described for larger organisms. Nevertheless, this trend was absent in this study for aquatic fungi along the altitudinal gradient. In Ecuador, the number of fungal taxa increased with the elevation and was strongly correlated with higher phosphates concentrations in water; whereas the moderate levels of nitrates influenced the Gausian curve depicted in Colorado.

On the other hand, the high similarity of bacteria between the tropical and the temperate latitude suggest that unicellular organisms display a cosmopolitan distribution. As was hypothesized, the temperature at lower altitudes was consistence with the raise in the number bacteria taxa in Ecuador and Colorado, despite this pattern was no find in a

broader scale, in which the diversity was similar between latitudes. The PCA analysis related temperature with the number of bacteria OTUs in Ecuador and Colorado, but in the same way the higher amounts of CPOM standing stock in the streams were highly correlated with this result.

In conclusion, the latitudinal pattern of species richness for larger organisms was no corroborated for microbial decomposers assessed in this study (aquatic fungi and bacteria), confirming that small organisms had a null relationship with the large scale patterns as has been reported in the literature (Hillebrand and Azovsky 2001; Hillebrand 2004) and rejecting the hypothesis that the latitude variability in temperature mirror the altitudinal gradient in species richness. The current results suggest that the number and the distribution of fungal and bacteria taxa, as well as the community assemblage depends of local environmental variables more than broadly factors that apparently influenced larger organisms, which confirming that small size organisms do not follow the pervasive species richness trend (Fuhrman et al. 2008). The lower dissimilarities recorded in the tropical streams along the altitudinal gradient suggest that the distribution of microorganisms nether respond to the temperature influenced as the main factor. Given that the thermal tolerance ranges were not measured specifically for representative species of bacteria and fungi in the studied sites, further research should be done in order to reveal the influence of temperature in the microorganisms distribution and its consequently effect in the microbial decomposition on leaf litter in streams.

To confirm the results recorded in the current study future studies should assess longer time of decomposition in the tropics, because earlier stages of the process might be underestimate the diversity and influencing the temporal community composition of decomposers.

4. GENERAL CONCLUSIONS AND PERSPECTIVES

1. In Ecuador, the decrease of microbial decomposition rates with elevation and the relationship between elevation and temperature strongly suggests that temperature is an important factor controlling litter decomposition. This was not the case for Colorado where fastest decomposition rate was attained at middle altitude. Litter decomposition was not correlated with temperature not with any of the measured variables. Further studies should be carried out in order to elucidate the reasons for these results. The difference between Colorado and Ecuador data could be related with nutrient availability. Since nitrates were ~30 fold higher in Colorado than in Ecuador, it is plausible that nutrients override the temperature effect on litter decomposition.

2. In terms of latitudinal comparisons, microbial decomposition rates were faster in Colorado than in Ecuador. Two factors could explain this difference. (1) As mentioned above, the higher nitrogen concentrations in Colorado than in Ecuador could be responsible for faster decomposition in Colorado. (2) Better quality if Colorado leaves (*Alnus incana*) than leaves from Ecuador (*A. acuminata*), which were tougher and had higher concentrations of polyphenolics.

3. Contrary to my expectations, the number of fungal taxonomic units along altitudinal gradient in Ecuador increased with elevation, whereas in Colorado the number of OTUs peaked at middle altitudes. In Ecuador differences in OTUs can be explained by differences in phosphorus (which increases with altitude). In Colorado, there was not a clear relationship between OTUs numbers and any of the environmental parameters, except moderate concentrations of nitrates and higher quantities of CPOM standing stock.

4. β Diversity (measured as dissimilarity relationships across altitudinal gradients) was lower in Ecuador than in Colorado, suggesting that Janzen proposal (thermal tolerance range of the organisms) may not apply to aquatic fungi. The taxonomical units of aquatic fungi in the tropical and the temperate latitude were similar. These results suggest that microbial community structure, as well as the diversity, respond to small scale variations in the environment such as nutrients concentrations in water and leaf substrates, more than the temperature. However, future research should assess the thermal tolerance of certain fungal species to corroborate that the temperature may not play a pivotal role constraining the aquatic fungi distribution.

5. As hypothesized, the number of bacteria taxonomical units decreased along the altitudinal gradient in Ecuador and Colorado. These results agree with the Island Theory if the taxa identity differs in the upper sites and with the temperature effect on diversity. However, higher quantities of CPOM standing stock and higher nitrates concentrations in water also influenced the diversity at lower sites at both gradients.

6. In terms of identity, contrary to my expectations, the similarity of bacteria taxa within Ecuador was higher than in Colorado. This result lesser the importance of the temperature as the main environmental factor controlling the distribution of bacteria taxa, because it suggest that bacteria in the tropical gradient occupy wider niches than in the temperate system. On the other hand, the number of bacteria OTUs and the identity of taxa between the two latitudes were not statistical different, suggesting that the latitudinal pattern in species richness observed in large individuals cannot be applied for the small size organisms, probably because of its wider capability to colonize broader areas and lower temperature variability in waters when compared with land systems.

7. Future works should assess the importance of aquatic fungi and specifically of aquatic hyphomycetes in the microbial decomposition in the tropical streams, as has been well stablished for the temperate systems. It would be interesting to determine the identity of aquatic hyphomycetes which are playing a pivotal role in the degradation of leaf litter in tropical streams combining morphological and molecular techniques, which can allow to identify specially the active species during the process assessing the RNA expression. Given that the structural compounds of the vegetative organic matter are degraded by the enzymatic activity, the quantification of the gene expression of certain enzymes such as cellulase or ligninase, could be tracked during the decomposition to understand this process at molecular level and linked with the identity of the taxa in the microbial community.

5. TABLES

Table 1. Geographical coordinates and temperatures (mean and range, recorded during the experiments) for the 10 streams in the tropical and temperate systems. E = Ecuador C = Colorado, USA. Average temperature (±SE).

Site code	Altitude (m asl)	Latitude	Longitude	Temperature (°C)	
E3847	3863	$0^0$14’51”S	78^0 9’60”W	6.9	(3.4-7.5)
E3415	3417	$0^0$13’47”S	78^0 7’46”W	9.3	(3.0-10.7)
E2838	2838	$0^0$14’46”S	78^0 0’21”W	8.4	(5.1-9.8)
E1805	1805	$0^0$15’20”S	$77^0$52’34”W	14.2	(13.2-14.9)
E1645	1645	$0^0$20’45”S	$77^0$49’22”W	17.4	(14.0-20.2)
C3200	3200	$40^0$42’60”N	$105^0$26’28”W	6.5	(3.1-13.3)
C2798	2798	$40^0$42’12”N	$105^0$35’46”W	7.1	(4.8-9.8)
C2590	2590	$40^0$56’40”N	$105^0$47’28”W	10.4	(6.2-17)
C2212	2212	$40^0$49’48”N	$105^0$43’32”W	11.9	(8.5-15.8)
C1992	1992	$40^0$37’30”N	$105^0$43’30”W	14.9	(10.7-20.6)

Table 2. Riparian vegetation and bed stream characterization for tropical and temperate streams (mean ± SD). The site code refers to the place (E= Ecuador, C = Colorado) and altitude.

Site	Canopy cover (%)	Substrate
E3847	0 ± 0.0	Boulder (66%); Lime (11%); Rock (9%)
E3415	94 ± 1.2	Cobble (64%); Pebble (29%); Grave (7%)
E2838	92 ± 1.0	Pebble (36%); Cobble (24%); Sand (14%); Gravel (14%)
E1805	93 ± 5.7	Cobble (28%); Pebble (25%); Grave (24%); Sand (16%)
E1645	96 ± 3.8	Cobble (32%); Pebble (30%); Gravel (15%); Sand (13%)
C3206	24 ± 0.7	N/A
C2798	68 ± 0.3	Sand (58%), Pebble (6%), Gravel (6%)
C2590	39 ± 1.3	Sand (47%), Pebble (30%), Gravel (12%)
C2212	76 ± 0.4	Sand (35%), Pebble (30%), Gravel (24%)
C1992	47 ± 1.1	Sand (41%), Pebble (24%), Gravel (18%)

N/A= No information available

Table 3. Physical and chemical parameters of the tropical sites (mean ± SD; n = 5)

	SITES				
	E3847	**E3415**	**E2838**	**E1805**	**E1645**
Conductivity µS/cm (25°C)	50.9 ± 12.1	32.6 ± 3.4	71.8 ± 16.5	124.9 ± 18.8	109.4 ± 11.7
pH	7.6 ± 0.3	7.9 ± 0.3	8.2 ± 0.3	8.3 ± 0.5	8.1 ± 0.1
Atmospheric pressure (hPa)	644.2 ± 1.0	676.5 ± 335.1	732.4 ± 0.8	822.4 ± 1.6	836.4 ± 1.5
Oxygen (%)	65 ± 2	68 ± 3	73 ± 2	81 ± 2	81 ± 3
Average flow m^3/s	0.05 ± 0.02	0.21 ± 0.17	0.18 ± 0.16	0.38 ± 0.46	0.08 ± 0.05
Depth (m)	0.21 ± 0.15	0.24 ± 0.17	0.20 ± 0.15	0.40 ± 0.55	0.18 ± 0.10
Width average (m)	0.74 ± 0.29	1.53 ± 0.14	3.16 ± 1.44	2.66 ± 0.59	0.55 ± 0.87
Phosphates (µg/L)	15.7 ± 7	13.1 ± 3.5	10.0 ± 4.2	5.6 ± 1.6	12.0 ± 2.5
Sulfates (µg/L)	3.2 ± 1.3	1.8 ± 1.3	3.0 ± 2.0	12.4 ± 3.1	3.6 ± 0.9
Nitrates (µg/L)	0.42 ± 0.08	0.38 ± 0.08	0.42 ± 0.04	0.38 ± 0.08	0.40 ± 0.07
Nitrites (µg/L)	0.0016 ± 8.9E-04	0.0008 ± 0.001	0.0024 ± 0.002	0.0014 ± 0.001	0.0022 ± 0.001
Ammonia (g/L)	0.060 ± 0.090	0.050 ± 0.062	0.048 ± 0.053	0.042 ± 0.053	0.092 ± 0.030

Table 4. Differences among streams for several environmental parameters. ANOVA (F) or Kruskal Wallys (H) when data was not normally distributed. Oxygen was subjected to an arc-sin transformation. Degrees of freedom (*df*) for all the parameters were equal to 4.

Parameter	Test	*P*	Comparisons
Water Temperature (°C)*	F= 158.88	<0.001	1645 > 1805; 2838; 3415; 3847 1805 > 2838; 3415; 3847 2838 > 3847 3415 > 3847
Conductivity (µS/cm)*	F=7.979	<0.001	1645 > 3415; 2838 1805 > 3415
pH	H=6.773	0.148	
Dissolved oxygen (%)*	F=19.568	<0.001	1645; 1805 > 2838; 3415; 3847 2838 > 3847
Discharge (m^3/s)	F=1.121	0.378	
Phosphates (µg/L)*	H=10.733	0.03	1805 < 3847
Nitrates (µg/L)	H=1600	0.809	
Nitrites (µg/L)	F=1.241	0.326	
Ammonia (µg/L)	H.4.937	0.294	
Sulfates*	F=26.43	<0.001	1805 > 1805; 2838; 3415; 3847
FPOM (g/m^2)	F=1.705	0.190	
CPOM(g/m^2)	F=1.476	0.249	

* Variables that significantly differed among streams

Table 5. Physical and chemical parameters collected once during the decomposition experiment for the temperate streams

	SITES				
	C3200	**C2798**	**C2590**	**C2212**	**C1992**
Conductivity µS/cm (25°C)	38	N/A	113.03	N/A	184
pH	N/A	N/A	8.38	N/A	7.7
Oxygen (%)	65.4	N/A	86.69	N/A	71
Depth (m)	0.10	0.14	0.14	0.06	0.10
Width (m)	0.80	2.25	1.10	1.30	1.40
Discharge (m^3/s)	0.026	0.023	0.022	0.009	0.004
Phosphates (µg/L)	7.192	10.720	2.098	6.999	N/A
Nitrates (µg/L)	5.836	12.653	4.951	15.704	50.9104
CPOM (g/m^2)	0.0011	0.0099	0.0008	0.0014	0.0011

Table 6. Decomposition rates (*k*), *p* values and R squares for each altitude applying the exponential decay model.

ID site	*k*	SE	*p*	R^2
E3847	0.0065	0.017	0.0001	0.50
E3415	0.0062	0.017	1E-05	0.57
E2838	0.0094	0.027	5E-07	0.69
E1805	0.0127	0.036	0	0.92
E1645	0.0144	0.040	2E-07	0.81
C3200	0.0197	0.030	3E-09	0.85
C2798	0.0231	0.056	3E-05	0.63
C2590	0.0453	0.172	1E-05	0.66
C2212	0.0187	0.036	5E-06	0.68
C1992	0.0240	0.043	0	0.90

Table 7. Initial litter quality of both *Alnus* leaves species used in this study (mean±SD) where all the variables were significantly different.

	Alnus acuminata Kunth	***Alnus incana (L.)***	
Quality parameters	**Mean**	**Mean**	***p***
P (%DM)	0.57 ± 0.26	1.61 ± 0.44	0.010
N (%DM)	3.64 ± 0.15	2.33 ± 0.17	2.71E-05
Polyphenolics (%DM)	27.88 ± 6.65	9.24 ± 1.21	0.002
Lignin (%DM)	49.67 ± 0.96	34.32 ± 1.30	1.66E-09
Specific leaf area (cm2 g-1)	402.75 ± 76.41	954.68 ± 277.05	0.00010

Unbalance t-test with two tails, $p \leq 0.05$

6. FIGURES

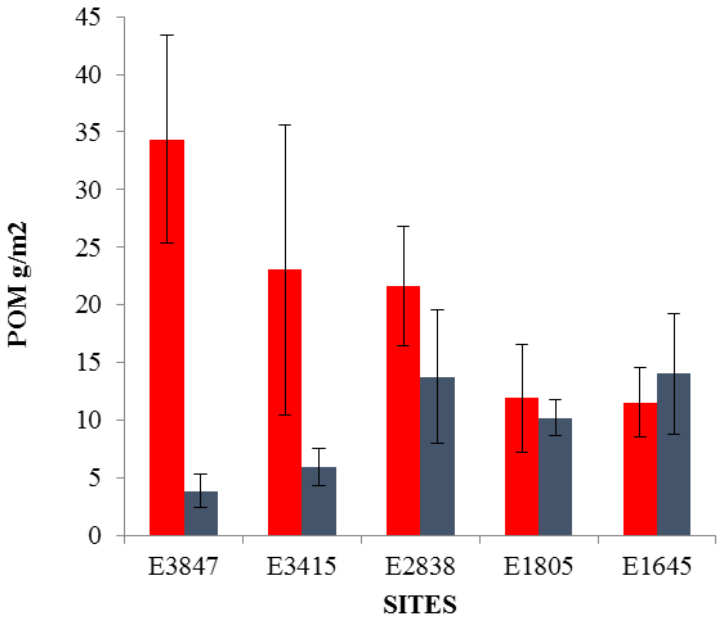


Figure 1. Mean of benthic fine (red bars) and coarse (blue bars) particulate organic matter (mean± SE) for the 5 tropical streams along the altitudinal gradient.

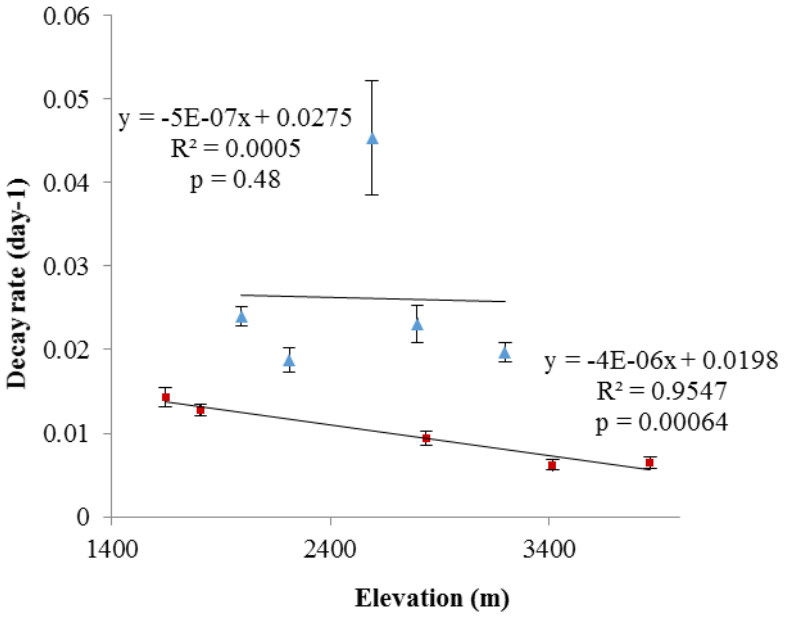


Figure 2. Breakdown (k/day) of *A. acuminate* (Ecuador, red squares) and *A. incana* (Colorado, blue triangles) along elevation gradients and respective regression parameters.

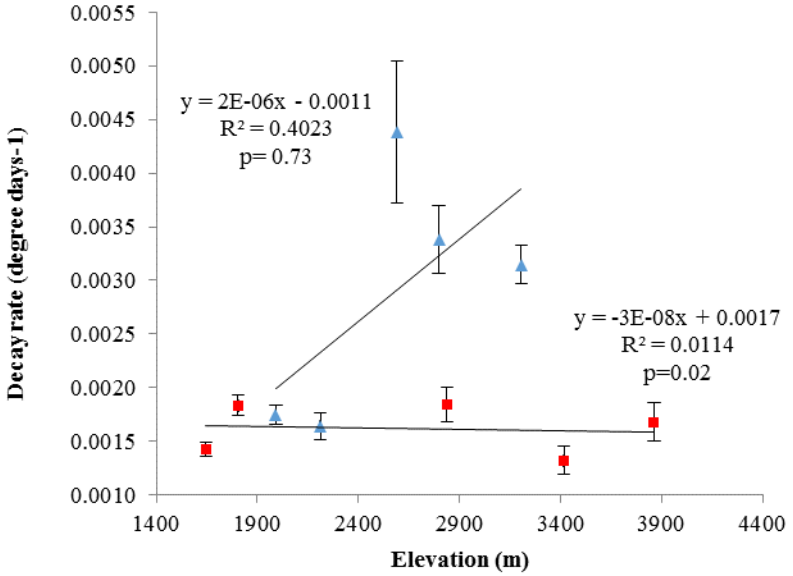


Figure 3. Decay rates expressed in terms of degree days related with the elevation for tropical and temperate latitudes. Equations, R squares and *p* values are included.

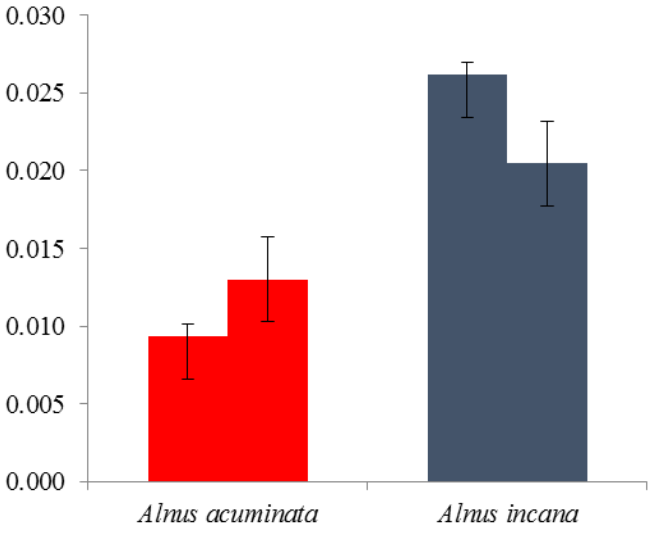


Figure 4. Decomposition rates of *Alnus acuminate* (red) and *Alnus incana* (blue) in their respective native range area (2013) and the second (2014) essay in the Ecuadorian site (E2838) to compare the differences of litter decomposition under the same environmental variables. Bars represent the mean ± SE.

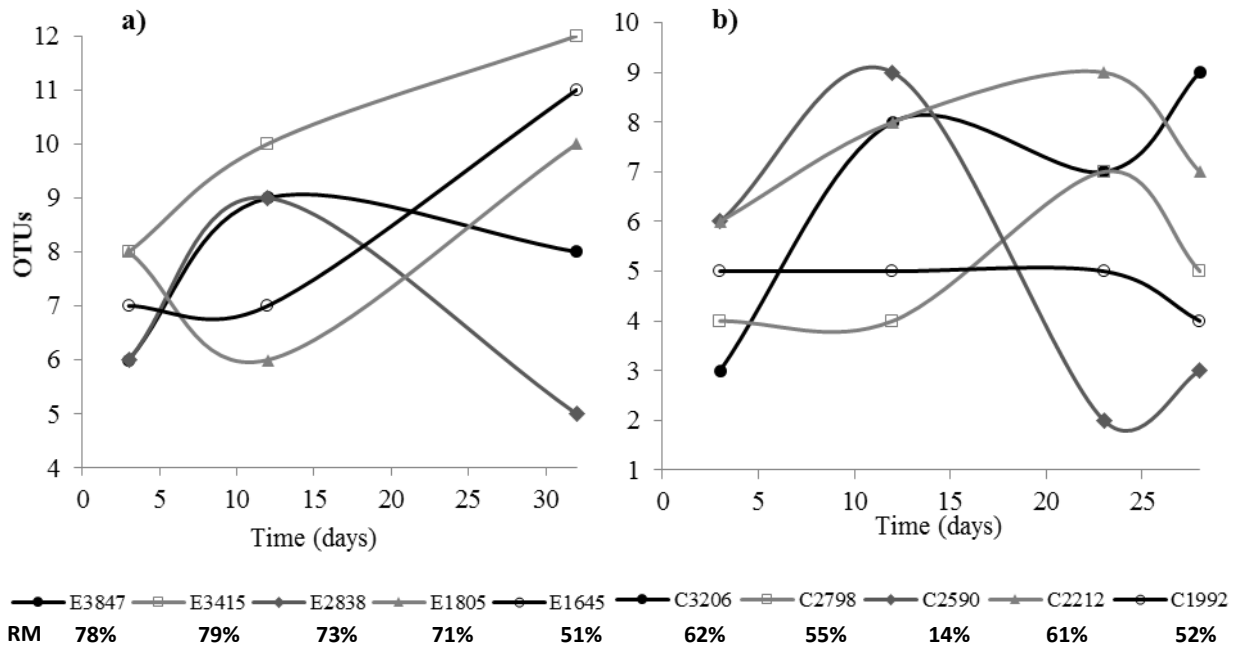


Figure 5. Temporal dynamic of aquatic fungi OTUs during leaf litter decomposition for a) Ecuador and b) Colorado streams. Percentages indicate the remaining mass (RM) for each sample after 32 and 28 days for both latitudes and further details of decomposition are described in the Table 6, Chapter 1.

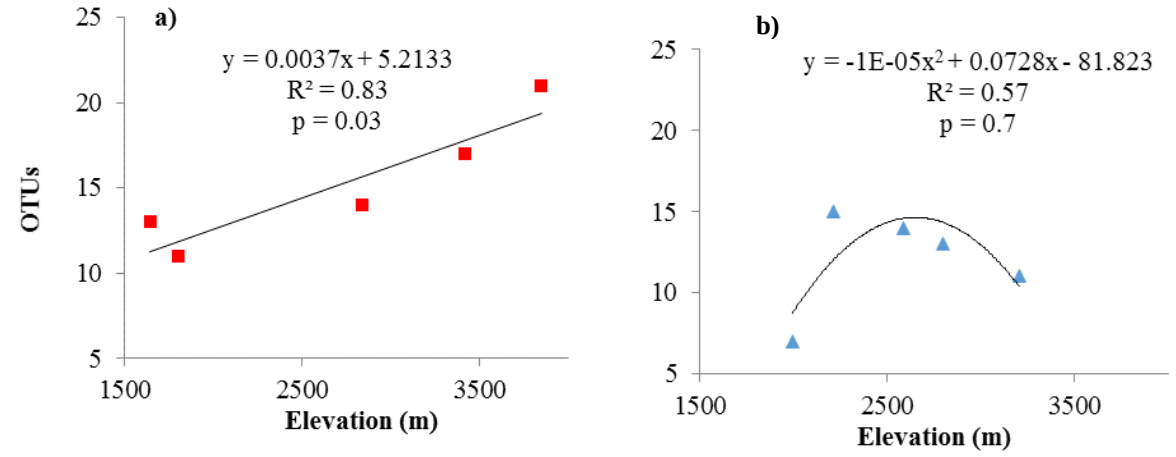


Figure 6. a) Linear regression between elevation *vs* number of aquatic fungi OTUs pooled by site for Ecuador and b) polynomial regression (order 2) for Colorado gradient. The number of bands were counted from the acrylamide gels detailed in the Annex 8.

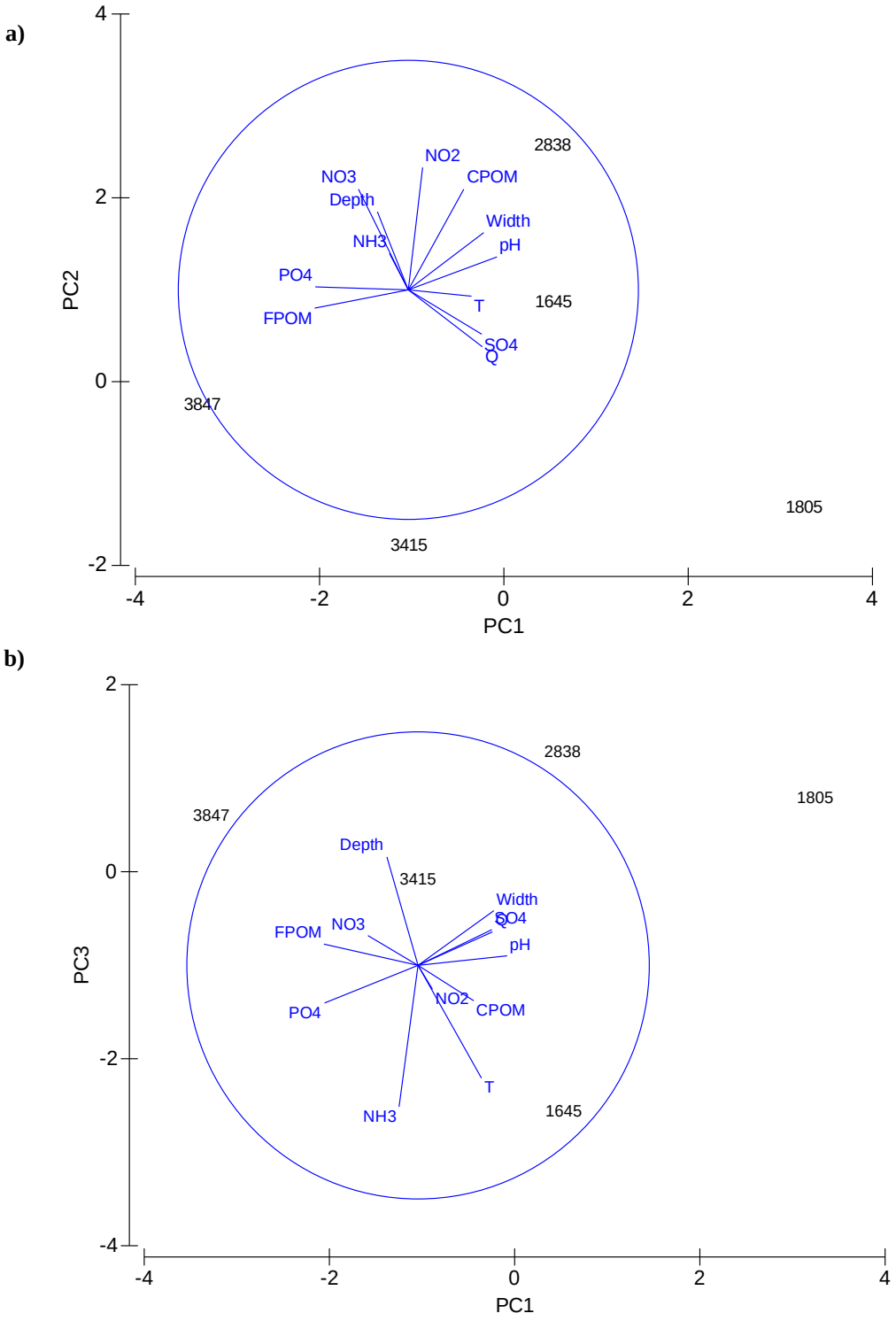


Figure 7. PCA calculated with the environmental variables collected from the five streams along the altitudinal gradient in Ecuador. Axes 1, 2 and 3 explained 92.9% of the total variability (Axis 1: 47.7%; Axis 2: 26%; Axis 3: 19.2%). a) Axis 1 and 2 and b) Axis 1 and 3. The first axis was positively related with higher pH values and negatively with higher phosphates concentrations in water and FPOM. The second axis was associated with higher levels of nitrites, nitrates and CPOM. The third axis was negatively related with temperature and ammonia and negatively with depth.

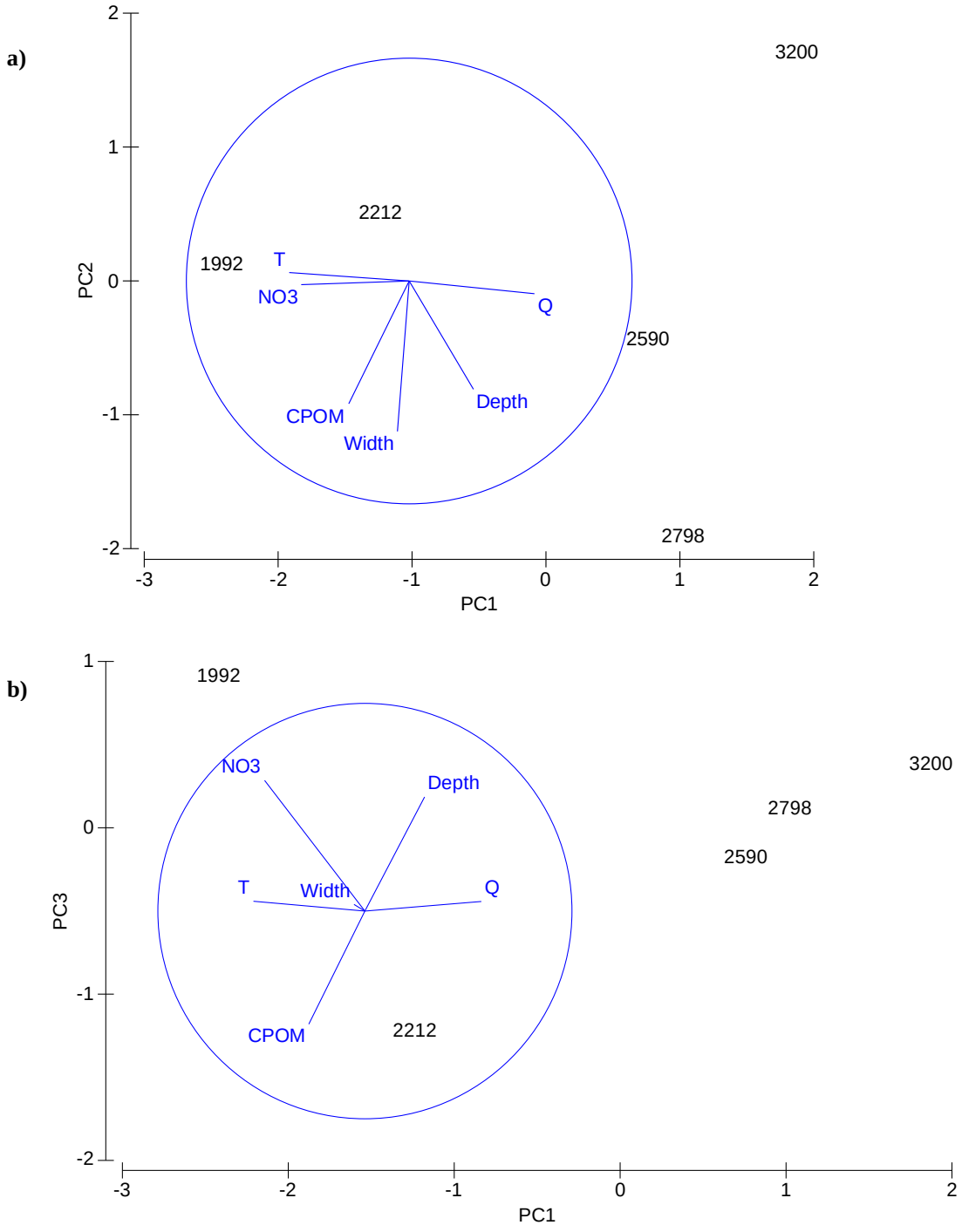


Figure 8. PCA analysis for Colorado streams with the environmental variables. The three principal axis of the PCA explained 91.8% of the biological variability (Axis 1: 52.2%; Axis 2: 29.2%; Axis 3: 10.4%). a) Axis 1 and 2 and b) Axis 1 and 3. The fist axis was positively related with discharge and negative related with temperature and nitrates. The second axis was negatively related with width, depth and CPOM. Axis 3 was mainly associated with nitrates.

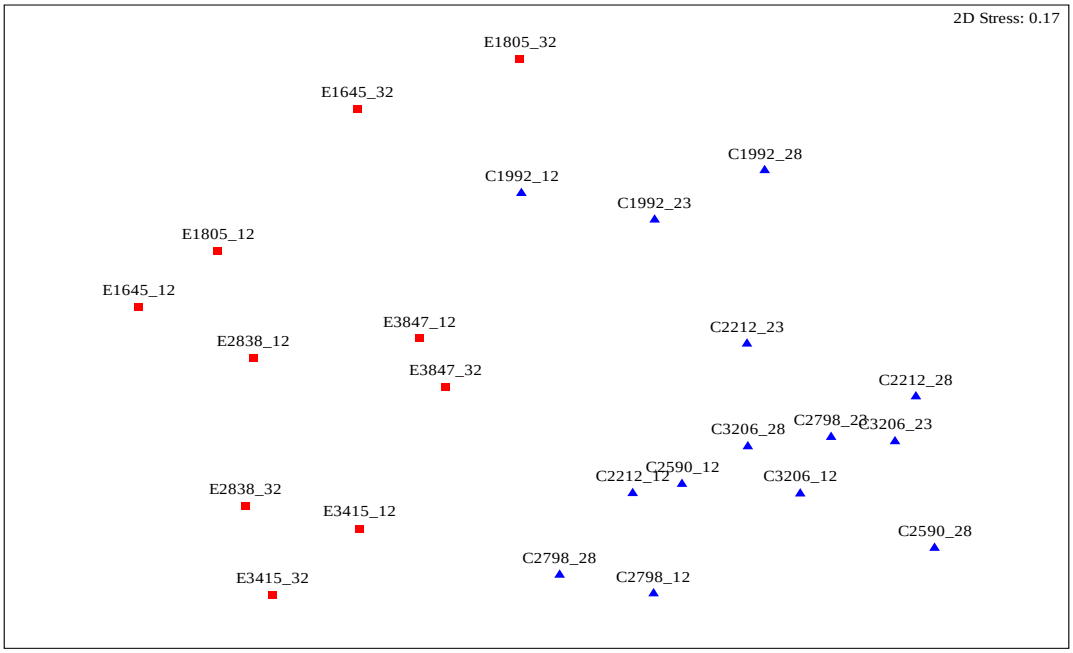


Figure 9. MDS performed with Bray-Curtis similarity separated the tropical (left; squares) and the temperate (right; triangles) sites (stress value = 0.17).

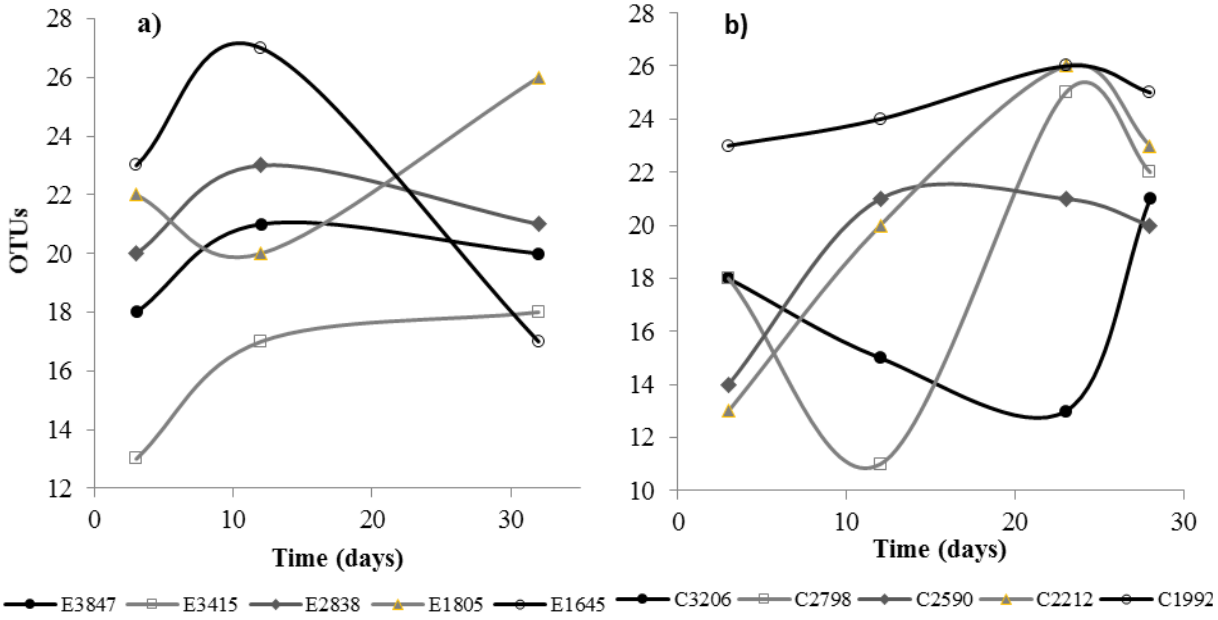


Figure 10. Temporal dynamic of bacteria communities for the 10 low order streams in a) Ecuador and b) Colorado over time.

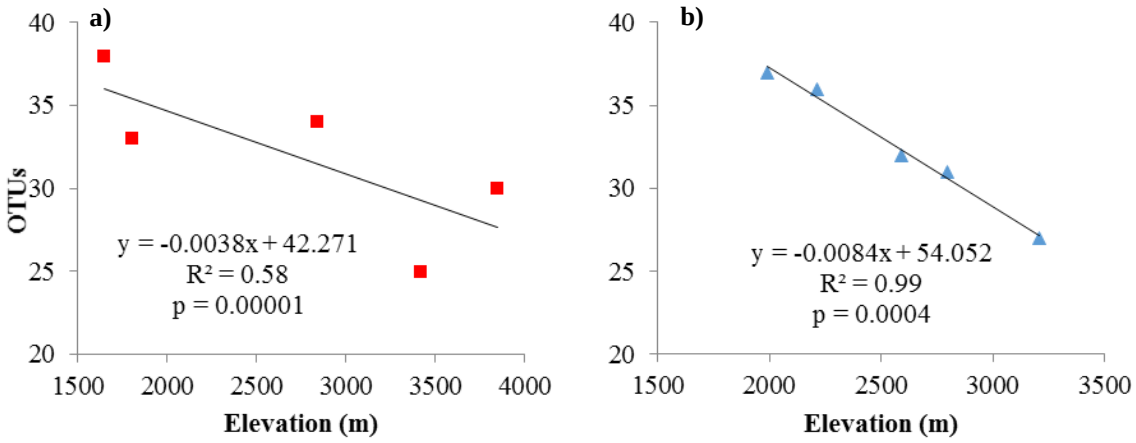


Figure 11. Relationship between the total number of bacteria OUT recorded in the a) tropical and b) temperate streams, during the three and four sampling dates, respectively. The number of bands, considered as OTUs are detailed in the Annex 14.

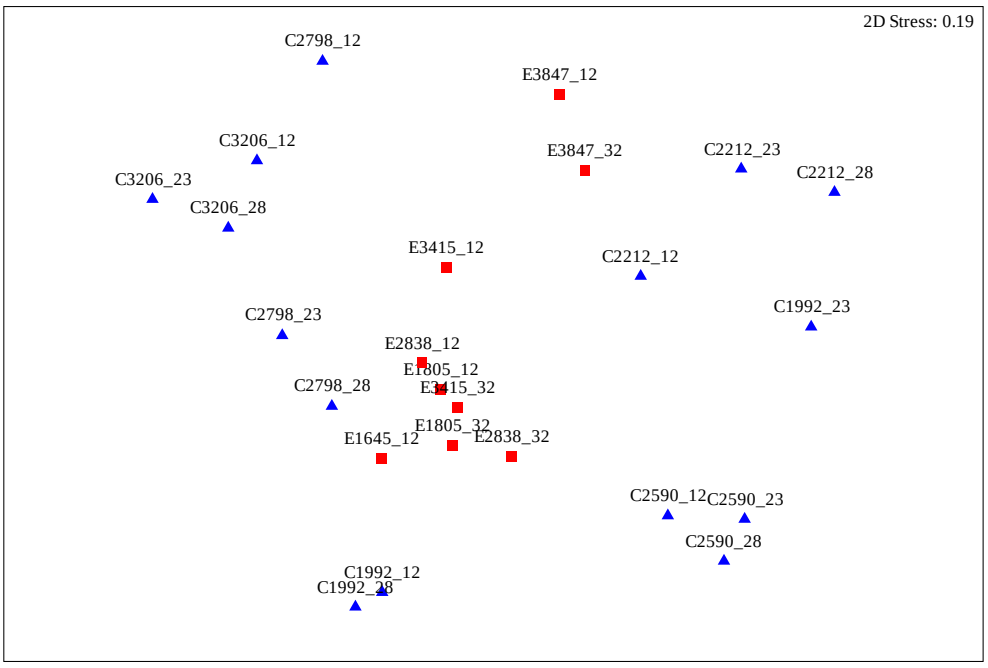
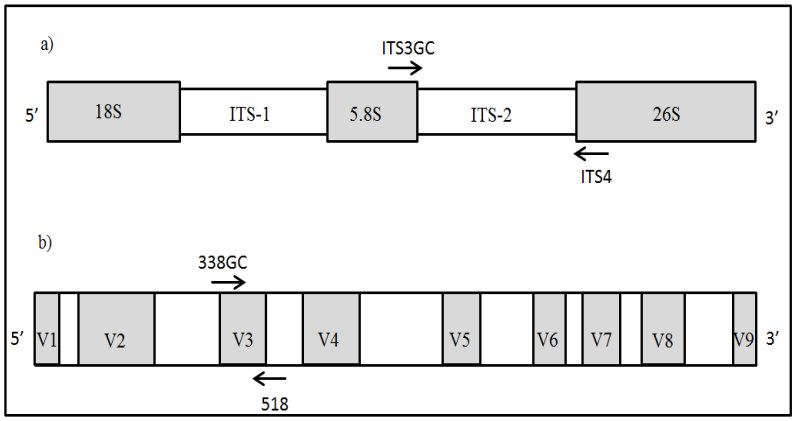


Figure 12. MDS performed with Bray-Curtis similarity among tropical (squares) and temperate (triangles) streams.

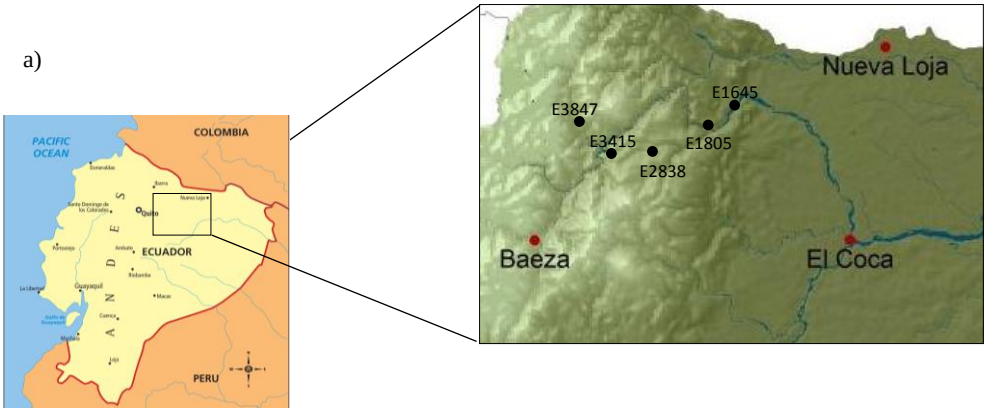
7. ANNEX

Annex 1. Representation of fungal and bacteria rRNA sequences used for molecular analyses. a) ITS region diagram based on the illustrations published by White et al. (1990) and Karp (2005). b) Variable regions within the 16S rRNA gene of *E.coli* as a model organism. The arrows depict the primer sets selected in this study to assess the fungal ITS2 region (ITS3GC/ ITS4) and the V3 region of bacteria (338GC/518) to determine their community assemblage.

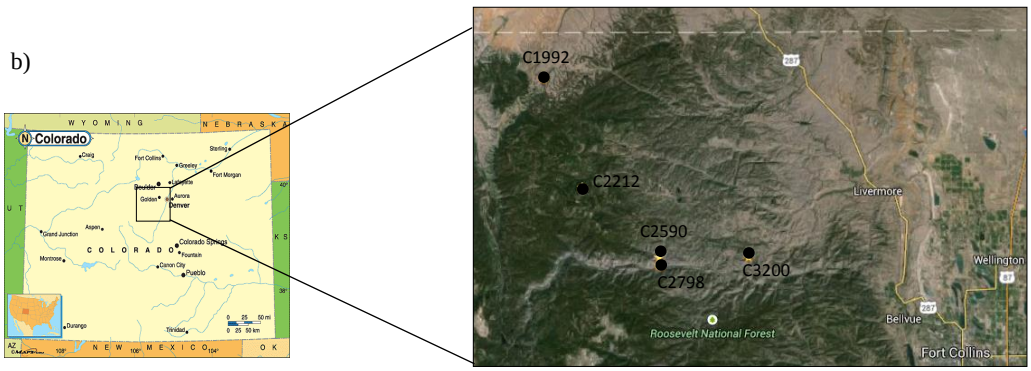


Annex 2. Map of the five head order streams a) Ecuador (tropical latitude) and b) Colorado (temperate latitude). The square within each figure (left) show the study area for each system, which is amplified (right) to visualize the streams along the altitudinal gradient where the study was carried out. Table 1 contains the site names and the geographical coordinates for each site.

a)



b)



Annex 3. IHF Scores for the 5 study sites are above 75%, meaning lower anthropogenic impact and a suitable environment for the benthic macroinvertebrates development.

	E3847	E3415	E2838	E1805	E1645
1.- Riffles inclusion	10	7	8	10	8
2.- Riffles frequency	10	10	10	10	10
3.- Substrate composition	17	17	20	17	20
4.- Regimes of speed/depth	10	10	10	10	10
5.- Shadow percentage in the river channel	5	10	10	10	7
6.- Heterogeneity elements (litter presence)	4	8	10	9	10
7.- Aquatic vegetation presence	25	25	11	20	15
TOTAL	81	87	79	86	80

Annex 4. Quality of riparian vegetation according the QBR (≥ 96 Very good; 76-95 = Good).

	E3847	E3415	E2838	E1805	E1645
1.- Riparian vegetation cover	25	25	17	23	15
2.- Riparian vegetation structure	20	25	25	25	25
3.- Vegetation cover quality	25	25	25	25	25
4.- Degree of fluvial channel naturalness	15	25	23	25	23
TOTAL	85	100	90	98	88

Annex 5. Protocol of Power Soil DNA Isolation Kit (MO BIO Laboratories, Inc.)

1. Place the sample into the PowerBead Tubes and gently vortex to mix it with the buffer contained in the tube. It will helps to protect the nucleic acid from degradation and will begin to dissolve humic acid.
2. Add 60 µl of Solution C1 that contains SDS and other disruption agents for the cell lysis. If the Solution C1 is precipitated, it must be heated to 60° C until the SDS gets dissolved and it can be used while it is still warm
3. Vortex the tubes in a horizontal flat-bed vortex pad (Mini-Tube Vortex) during 10 min to homogenize the content inside the tube and to complete the cell lysis
4. Centrifuge tubes at 10,000 x g for 30 seconds at room temperature
5. Transfer the supernatant (around 450 µL) to a clean 2 mL collection tube and add 250 µL of Solution C2. This reagent contains a solution called Inhibitor Removal Technology (IRT) that precipitates the inorganic and non DNA organic material such as humic substances, cell debris and proteins which could interfere in the subsequent DNA applications
6. Vortex for 5 seconds and incubate the samples at 4°C for 5 minutes
7. Recovered around 500 µL of the supernatant to a clean 2 mL Eppendorf tube avoiding the contact of the pellet which contains the precipitated substances
8. Add 200 µL of Solution C3 and invert the tubes. This solution is also an IRT reagent that precipitates for second time the non-DNA organic substance as well as inorganic compounds.
9. Incubate again the samples at 4°C for 5 minutes

10. Centrifuge the tubes at room temperature for 1 minute at 10,00 x g
11. Transfer around 650 µL of supernatant to a clean Eppendorf tube
12. Add 1200 µL of Solution C4 to the supernatant and vortex 5 seconds to allow the DNA binding to the membrane
13. Load around 670 µL of the previous solution into the Spin Filter and centrifuge at 10,000 x g for 1 minute at room temperature. Repeat this step three times until all the solution of each sample get processed
14. To remove residual salts, humic acid and other contaminants the next step is going to wash the DNA
15. Add 500 µL of Solution C5 and centrifuge at room temperature for 30 seconds at 10,000 x g to remove residual salts from the DNA.
16. Discard the flow and centrifuge again 1 minute under the same conditions above to complete the wash step using ethanol contained in the Solution C5 (wash solution)
17. Place the Spin Filter in a clean 2 mL collection tube avoiding the contact with the wash solution
18. Add 100 µL of Solution C6 in the center of the filter membrane to elude effectively the DNA from the silica membrane. This solution contains 10 mM Tris which selectively released the DNA from the membrane.
19. Centrifuge 30 seconds under the same conditions described above and discard the Spin Filter
20. Storage the DNA recovered at -20° C

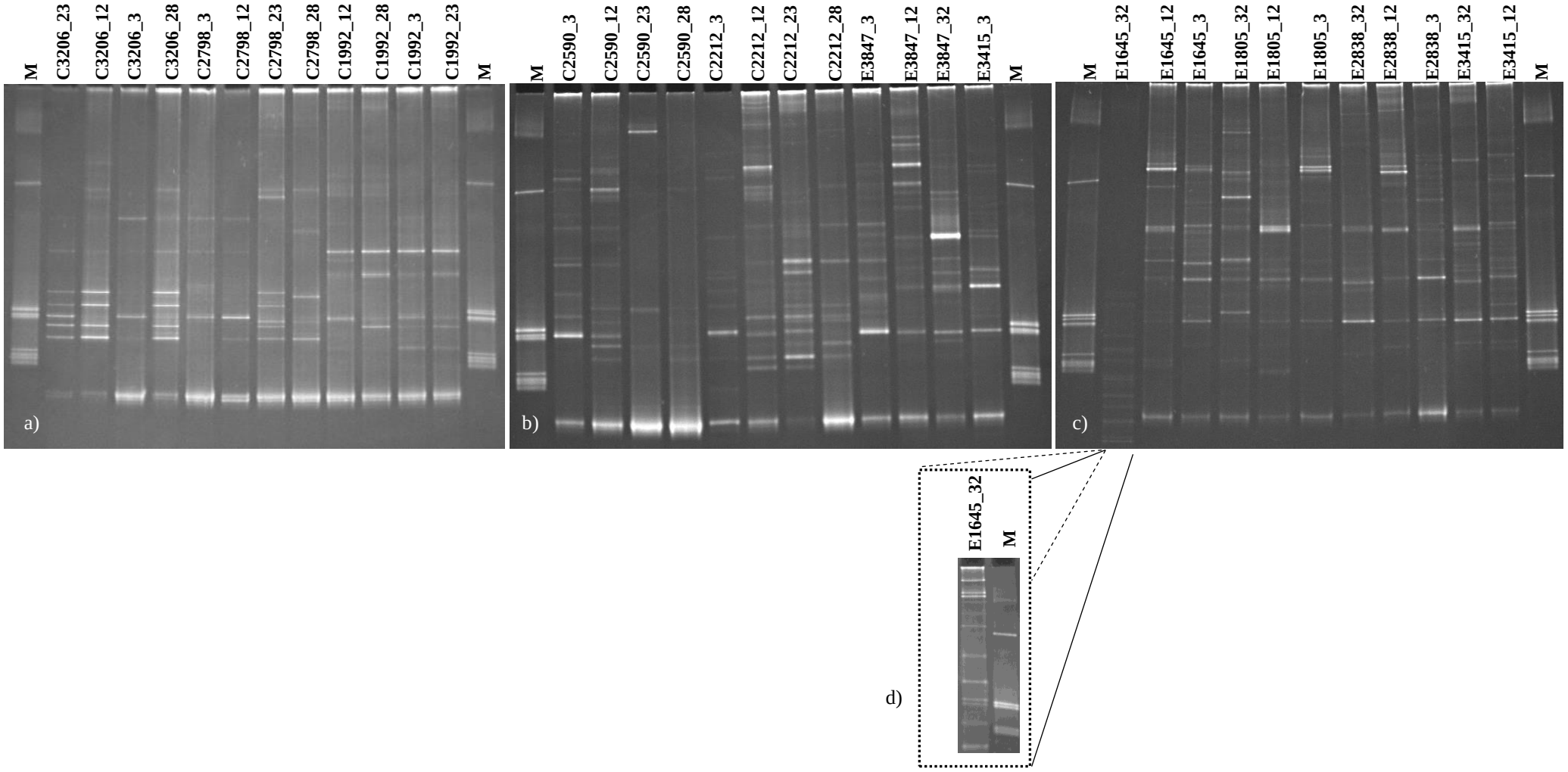
Annex 6. Reagent concentrations for acrylamide matrix denaturant gradient. LD = Low density; HD = High density

	Fungi		**Bacteria**	
	LD	**HD**	**LD**	**HD**
	30%	**70%**	**40%**	**70%**
Urea	2.016 g	4.705 g	2.688 g	4.705 g
Bis-acrylamide	3200 µL	3.2 µL	3200 µL	3200 µL
TAE 50X	320 µL	320 µL	320 µL	320 µL
Formamide	192 µL	448 µL	2.56 µL	448 µL
H_20 deionized	up to 16 mL	up to 16 mL	up to 16 mL	up to 16 mL
TEMED	14.4 µL	14.4 µL	14.4 µL	14.4 µL
APS	144 µL	144 µL	144 µL	144 µL

Annex 7. Species of aquatic hyphomycetes and bacteria amplified from pure cultures and used as reference bands for the gel alignment

Aquatic hyphomycetes	**Bacteria**
Dimosphora folicola	*Proteus vulgaris*
Angillispora filiformis	*Staphylococcus aureus*
Triphosphorus acuminatus	*Pseudomona putida*
Flagellospora renicillioides	*Eschericha coli*
Alatospora pulchella	
Tricladium splendens	
Articulospora tetracladia	
Clavatospora longibrachiata	
Tetrachaetum elegans	

Annex 8. DGGE gels (a-c) with the fungal communities' fingerprinting during the decomposition in the 10 low order streams. M = Mix of pure fungal cultures used for the alignment. The sample E1645_32 was amplified twice due to PCR inconveniences. The figure 6.d shows the second amplification.



Annex 9. Eigenvectors or coefficients in the linear combinations of variables for the five axis determined in the PCA for Ecuador. Values with r > | 0.4| in bold.

Variable	PC1	PC2	PC3	PC4	PC5
T	**0.274**	**-0.027**	-0.483	**0.191**	**-0.038**
pH	**0.385**	**0.144**	**0.041**	**-0.308**	-0.459
Q	**0.322**	**-0.248**	**0.143**	0.442	**-0.056**
Width	**0.328**	**0.25**	**0.234**	**-0.275**	**-0.172**
Depth	**-0.135**	**0.34**	0.464	**0.213**	**0.039**
CPOM	**0.241**	0.439	**-0.152**	**-0.132**	**0.02**
FPOM	-0.407	**-0.079**	**0.091**	**0.119**	**-0.305**
PO4	-0.404	**0.013**	**-0.161**	**-0.059**	-0.711
SO4	**0.319**	**-0.193**	**0.153**	0.535	**-0.342**
NO3	**-0.217**	0.439	**0.128**	**0.329**	**0.129**
NO2	**0.062**	0.534	**-0.102**	**0.278**	**-0.137**
NH3	**-0.082**	**0.159**	-0.606	**0.213**	**0.051**

Annex 10. Correlation coefficients (r) calculated between the scores determined by the five axis of the PCA for Ecuadorian streams and the total number of OTUs for microbial decomposers (aquatic fungi and bacteria).

		SCORES				
	Sample	**Ax 1**	**Ax 2**	**Ax 3**	**Ax 4**	**Ax 5**
	3847	-3.27	-0.257	0.593	0.996	-4.96E-16
	3415	-1.04	-1.79	-0.0886	-1.3	4.48E-16
	2838	0.522	2.56	1.28	-0.534	-3.85E-16
	1805	3.25	-1.38	0.785	0.638	4.09E-16
	1645	0.533	0.857	-2.57	0.205	-9.71E-17
r coefficient	**Aquatic Fungi**	-0.97	-0.14	0.17	0.44	-0.46
	Bacteria	0.46	0.64	-0.43	0.44	-0.36

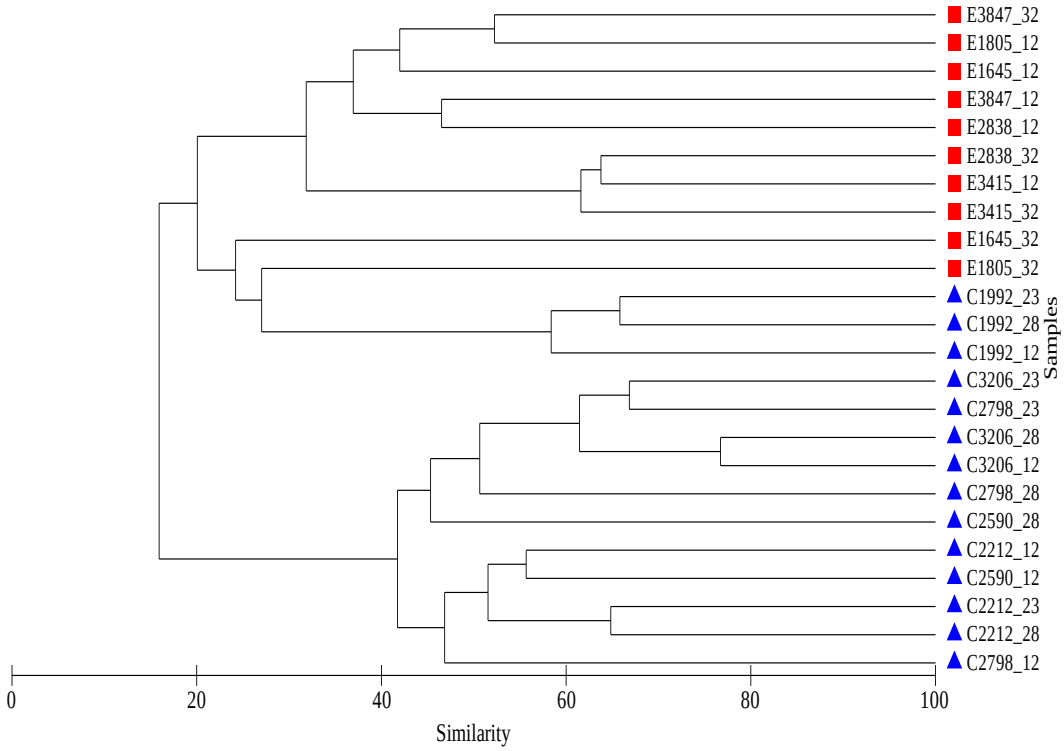
Annex 11. Principal Component Scores determined by the PCA on the environmental variables performed for the Colorado streams.

		SCORES				
	Sample	**Ax 1**	**Ax 2**	**Ax 3**	**Ax 4**	**Ax 5**
	3200	1.87	1.71	0.379	0.3	-1.11E-16
	2798	1.02	-1.9	0.112	0.617	2.78E-16
	2590	0.759	-0.433	-0.18	-1.19	3.47E-17
	2212	-1.24	0.509	-1.22	0.301	5.55E-17
	1992	-2.42	0.12	0.91	-3.25E-02	0
r coefficient	**Aquatic Fungi**	0.40	-0.21	-0.88	-0.11	0.35
	Bacteria	-0.91	-0.19	-0.18	-0.09	0.15

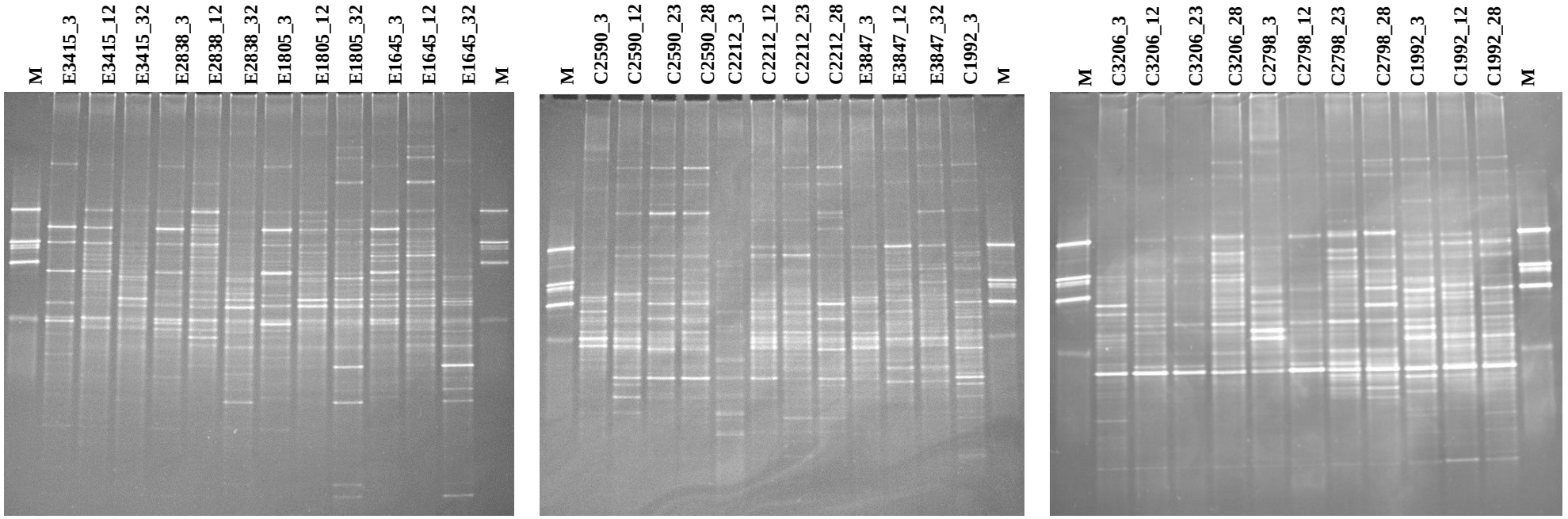
Annex 12. Eigenvectors for the five principal axes calculated by the PCA analysis with the environmental variables for Colorado. Values with r > | 0.4| in bold.

Variable	PC1	PC2	PC3	PC4	PC5
Depth	**0.288**	-0.486	**0.55**	-0.528	**0.282**
Width	**-0.053**	-0.675	**0.032**	0.627	**-0.171**
Q	0.562	**-0.057**	**0.046**	**-0.079**	-0.774
NO3	-0.485	**-0.016**	0.629	**0.181**	**-0.134**
CPOM	**-0.271**	-0.551	-0.545	**-0.33**	**0.008**
T	-0.538	**0.038**	**0.047**	-0.424	-0.523

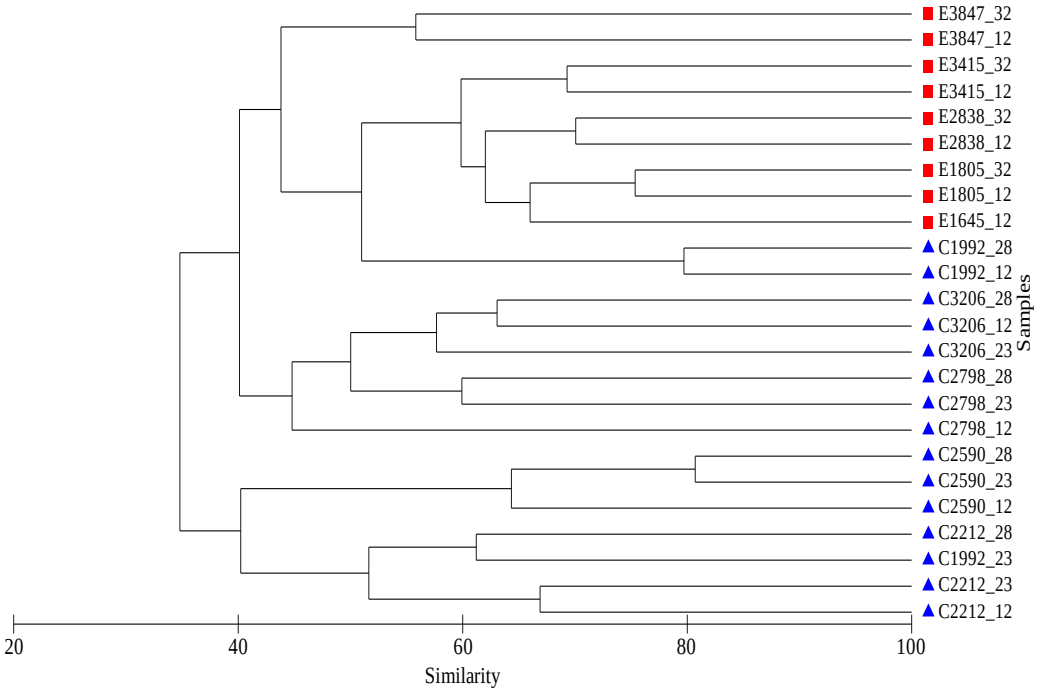
Annex 13. Cluster analyses with Bray-Curtis similarity and UPGM group linkage method of 24 samples of aquatic fungi from the tropical (squares) and the temperate (triangles) streams.



Annex 14. Bacteria community fingerprint displayed in the DGGE gels for samples in the tropical and temperate streams along the altitudinal gradient recovered different dates over the litter breakdown. M = Mix of pure bacteria cultures used for the alignment. Samples' identification follows the same criteria as explained for the fungal fingerprinting.



Annex 15. Bacteria dendogram with Bray- Curtis similarity of 24 samples from Ecuador (E; squares) and Colorado (C; triangles).



Annex 16. Species of aquatic hyphomycetes recorded in the five low order streams in Ecuador. The morphological identification of spores was induced incubating leaf pieces of *Alnus acuminate* Kunth. retrieved from fine mesh bags after 30 days of submersion in the streams (1st of February to 3 of March of 2014). Leaves were air dried and transported from Ecuador to Portugal. Ones in the laboratory, the leaves were incubated at 15° C during 24 h in glass flasks containing 150 mL of distilled (five replicates for each stream) and autoclaved water with aeration, followed by a period of incubation between 48 to 72 hours after which the spores were dyed with 0.08% trypan blue in 60% lactic acid, filtered (0.5 µm pore size, Millipore) and observed under the microscope. The water was renewed after the first 24 h and after 72 h when the spores in the samples were absent.

Aquatic hyphomycete species	Altitude
Heliscus lugdunensis	3847; 3415; 2838; 1805; 1605
Lemonniera spp.	3847; 3415; 2838; 1805; 1605
Cilindrocarpus	3847
Alatospora pulchela	3847
Varicosporium spp	3415
Trichladium splendes	3415
Clavatospora longibrachiata	3415
Lunulospora curvula	2838
Tetrachaetum elegans	2838; 1805; 1645
Tetracladium marchalianum	2838; 1605

8. REFERENCES

Abelho, Manuela. 2005. “Extration and Quantification of ATP as a Measure of Microbial Biomass.” In *Methods to Study Litter Decomposition: A Practica Guide*, edited by Manuel A.S. Graça, Felix Barlocher, and Mark O. Gessner, 223–29. The Netherlands: Springer.

Acosta, Raúl, Blanca Ríos, Maria Rieradevall, and Narcís Prat. 2009. “Propuesta de Un Protocolo de Evaluación de la Calidad Ecológica de Ríos Andinos (CERA) y su Aplicación a Dos Cuencas en Ecuador y Perú.” *Limnetica* 28 (1): 35–64.

American Public Health Association. 1998. *Standard Methods for the Examination of Water and Wastewater*. 20th ed. Washington DC.

Andrew, Nigel R., and Lesley Hughes. 2005. “Herbivore Damage along a Latitudinal Gradient: Relative Impacts of Different Feeding Guilds.” *Oikos* 108 (1): 176–82.

Baldy, Virginie, Mark Gessner, and Eric Chauvet. 1995. “Bacteria, Fungi and the Breakdown of Leaf Litter in a Large River.” *OIKOS* 74: 93–102.

Bärlocher, Felix. 2005. “Freshwater Fungal Communities.” *The Fungal Community: Its Organization and Role in the Ecosystem*, no. JANUARY 2005: 39–59.

Bärlocher, Felix. 2007. “Molecular Approaches Applied to Aquatic Hyphomycetes.” *Fungal Biology Reviews* 21: 19–24.

Bärlocher, Felix.. 2010. “Molecular Approaches Promise a Deeper and Broader Understanding of the Evolutionary Ecology of Aquatic Hyphomycetes.” *Journal of the North American Benthological Society* 29 (3): 1027–41.

Bärlocher, Felix, and Miranda Corkum. 2003. “Nutrient Enrichment Overwhelms Diversity Effects in Leaf Decomposition by Stream Fungi.” *Oikos* 101 (November 2002): 247–52.

Bärlocher, Felix, and Ludmila Marvanová. 2010. “Aquatic Hyphomycetes (Deuteromycotina) of the Atlantic Maritime Ecozone.” In *Species Diversity in the Atlantic Maritime Ecozone*.

Belliveau, Michel J R, and Felix Bärlocher. 2005. “Molecular Evidence Confirms Multiple Origins of Aquatic Hyphomycetes.” *Mycological Research* 109 (December): 1407–17.

Bengtsson, Göran. 1992. “Interactions between Fungi, Bacteria and Beech Leaves in a Stream Microcosm.” *Oecologia* 89: 542–49.

Boulton, a, S Findlay, P Marmonier, E Stanley, and H Valett. 1998. “The Functional Significance of the Hyporheic Zone in Streams and Rivers.” *Annual Review of Ecological Systems* 29: 59–81.

Boyero, Luz, Richard G Pearson, Mark O Gessner, Leon a Barmuta, Verónica Ferreira, Manuel A.S. Graça, David Dudgeon, et al. 2011. “A Global Experiment Suggests Climate Warming Will Not Accelerate Litter Decomposition in Streams but Might Reduce Carbon Sequestration.” *Ecology Letters* 14 (3): 289–94.

Brown, James, James Gilloly, Andrew Allen, Van M Svagae, and Geoffrey West. 2004. “Perspectives: Toward a Metabolic Theory of Ecology.” *Ecology* 85 (7): 1771–89.

Brown, James H. 2014. “Why Are There so Many Species in the Tropics?” *Journal of Biogeography* 41: 8–22.

Bruns, Thomas, Thomas White, and Jhon Taylor. 1991. “Molecular Evolution of Fungi.” *Annual Review of Ecology and Systematics* 22: 525–264.

Canhoto, Cristina, and Manuel A.S. Graca. 1996. “Decomposition of Eucalyptus Globulus Leaves and Three Native Leaf Species (Alnus Glutinosa, Castanea Sativa and Quercus Faginea) in a Portuguese Low Order Stream.” *Hydrobiologia* 333: 79–85.

Capps, Krista a., Manuel a. S. Graça, Andrea C. Encalada, and Alexander S. Flecker. 2011. “Leaf-Litter Decomposition across Three Flooding Regimes in a Seasonally Flooded Amazonian Watershed.” *Journal of Tropical Ecology* 27 (02): 205–10.

Case, Rebecca J., Yan Boucher, Ingela Dahllöf, Carola Holmström, W. Ford Doolittle, and Staffan Kjelleberg. 2007. “Use of 16S rRNA and rpoB Genes as Molecular Markers for Microbial Ecology Studies.” *Applied and Environmental Microbiology* 73 (1): 278–88.

Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, et al. 2007. “Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget.” *Ecosystems* 10 (1): 172–85.

Coley, Phyllis D, and J.A. Barone. 1996. “Herbivory and Plant Defenses in Tropical Forests.” *Annual Review of Ecology and Systematics* 27 (1): 305–35.

Dangles Olivier, Mark O. Gessner, Francois Guerold, and Eric Chauvet. 2004. “Impacts of Stream Acidification on Litter Breakdown: Implications for Assessing Ecosystem Functioning.” *Journal of Applied Ecology* 41 (2): 365–78.

Das Mitali, Todd V. Royer, and Laura G. Leff. 2007. “Diversity of Fungi, Bacteria, and Actinomycetes on Leaves Decomposing in a Stream.” *Applied and Environmental Microbiology* 73 (3): 756–67.

Dodds. Walter. 2002. “Microbes and Plants.” In *Freshwater Ecology: Concepts and Environmental Applications*. Kansas State University. San Diego: Academic Press.

Dos Santos Fonseca, André Luiz, Irineu Bianchini, Cristiane Marques Monteiro Pimenta, Cássio Botelho Pereira Soares, and Norberto Mangiavacchi. 2013. “The Flow Velocity as Driving Force for Decomposition of Leaves and Twigs.” *Hydrobiologia* 703 (1): 59–67.

Duarte, Sofia, Fernanda Cássio, and Cláudia Pascoal. 2007. “Denaturing Gradient Gel Electrophoresis (DGGE) in Microbial Ecology – Insights from Freshwaters.” In *Gel Electorphoresis-Principles and Basics*, edited by Sameh Magdeldin. INTECH. www.interchopen.com.

Duarte, Sofia, Isabel Fernandes, Maria João Nogueira, Fernanda Cássio, and Cláudia Pascoal. 2013. “Temperature Alters Interspecific Relationships among Aquatic Fungi.” *Fungal Ecology* 6 (3): 187–91.

Duarte, Sofia, Cláudia Pascoal, Artur Alves, António Correia, and Fernanda Cássio. 2010. “Assessing the Dynamic of Microbial Communities during Leaf Decomposition in a Low-Order Stream by Microscopic and Molecular Techniques.” *Microbiological Research* 165 (5): 351–62.

Duarte, Sofia, Cláudia Pascoal, Fernanda Cássio, and Felix Bärlocher. 2006. “Aquatic Hyphomycete Diversity and Identity Affect Leaf Litter Decomposition in Microcosms.” *Oecologia* 147 (4): 658–66.

Duarte, Sofia, Sahadevan Seena, Felix Bärlocher, Fernanda Cássio, and Cláudia Pascoal. 2012. “Preliminary Insights into the Phylogeography of Six Aquatic Hyphomycete Species.” *PloS One* 7 (9): e45289.

Dudgeon, David. 2008. *Tropical Stream Ecology*. Amsterdam: Academic Press, Elsevier.

Farjalla, Vinicius, Francisco Esteves, Reinaldo Bozelli, and Fábio Ronald. 2002. “Nutrient Limitation of Bacterial Production in Clear Water Amazonian Ecosystems.” *Hydrobiologia* 489: 197–205.

Fellman, Jason B., Neil E. Pettit, Jesse Kalic, and Pauline F. Grierson. 2013. “Influence of Stream–floodplain Biogeochemical Linkages on Aquatic Foodweb Structure along a Gradient of Stream Size in a Tropical Catchment.” *Freshwater Science* 32 (1): 217–29.

Fernandes, Isabel, Cláudia Pascoal, Helena Guimarães, Rute Pinto, Inês Sousa, and Fernanda Cássio. 2012. “Higher Temperature Reduces the Effects of Litter Quality on Decomposition by Aquatic Fungi.” *Freshwater Biology* 57 (11): 2306–17.

Fernandes, Isabel, Begüm Uzun, Cláudia Pascoal, and Fernanda Cássio. 2009. “Responses of Aquatic Fungal Communities on Leaf Litter to Temperature-Change Events.” *International Review of Hydrobiology* 94 (4): 410–18.

Ferreira, Verónica, Bastien Castagneyrol, Julia Koricheva, Vladislav Gulis, Eric Chauvet, and Manuel a S Graça. 2014. “A Meta-Analysis of the Effects of Nutrient Enrichment on Litter Decomposition in Streams.” *Biological Reviews*.

Ferreira, Verónica, and Eric Chauvet. 2011a. “Synergistic Effects of Water Temperature and Dissolved Nutrients on Litter Decomposition and Associated Fungi.” *Global Change Biology* 17 (1): 551–64.

Ferreira, Verónica, and Eric Chauvet.. 2011b. “Future Increase in Temperature More than Decrease in Litter Quality Can Affect Microbial Litter Decomposition in Streams.” *Oecologia* 167 (1): 279–91.

Ferreira, Verónica, Andrea C. Encalada, and Manuel A.S. Graça. 2012. “Effects of Litter Diversity on Decomposition and Biological Colonization of Submerged Litter in Temperate and Tropical Streams.” *Freshwater Science* 31 (3): 945–62.

Ferreira, Verónica, and Manuel A.S. Graça. 2006. “Do Invertebrate Activity and Current Velocity Affect Fungal Assemblage Structure in Leaves?” *International Review of Hydrobiology* 91 (1): 1–14.

Ferreira, Verónica, Vladislav Gulis, and Manuel A.S. Graça. 2006. “Whole-Stream Nitrate Addition Affects Litter Decomposition and Associated Fungi but Not Invertebrates.” *Oecologia* 149 (4): 718–29.

Ferreira, Verónica, Vladislav Gulis, Cláudia Pascoal, and Manuel A.S. Graça. 2014. “Stream Pollution and Fungi.” In *Freshwater Fungi and Fungal-like Organisms (Marine and Freshwater Botany)*, edited by Jones Gareth, Kevin Hyde, and Ka-Lai Pang, 389–412. Berlin/Boston: Walter de Gruyter GmbH.

Fierer, Noah, and Robert B Jackson. 2006. “The Diversity and Biogeography of Soil Bacterial Communities.” *Proceedings of the National Academy of Sciences of the United States of America* 103 (3): 626–31.

Fierer, Noah, Christy M. Mccain, Patrick Meir, Michael Zimmermann, Joshua M. Rapp, Miles R. Silman, and Rob Knight. 2011. “Microbes Do Not Follow the Elevational Diversity Patterns of Plants and Animals.” *Ecology* 92 (4): 797–804.

Fierer, Noah, Jennifer L Morse, Sean T Berthrong, Emily S Bernhardt, and Robert B Jackson. 2007. “Environmental Controls on the Land-Scale Biogeography of Stream Bacterial Communities.” *Ecology* 88: 2162–73.

Fisher, S.G., and G.E. Likens. 1973. “Energy Flow in Bear Brook, New Hampshire: An Alternative Approach to Stream Metabolism Ecological Monographs,” 43:421–39.

Fuhrman, Jed. 2009. “Microbial Community Structure and Its Functional Implications.” *Nature* 459 (May): 193–99.

Fuhrman, Jed, Joshua Steele, Michael S. Schwalbach, Ian Hewson. 2008. “A Latitudinal Diversity Gradient in Planktonic Marine Bacteria.” *Proceedings of the National Academy of Sciences of the United States of America* 105: 7774–78.

Garnett, Heather, Felix Barlocher, and Donna Giberson. 2000. “Mycological Society of America Seasonal in Catamaran Brook : Colonization Dynamics , Aquatic Hyphomycetes and Logging Effects Patterns ,.” *Mycologia* 92 (1): 29–41.

Gaston, K J. 2000. “Global Patterns of Biodiversity.” *Nature* 405 (6783): 220–27.

Geraldes, Paulo, Cláudia Pascoal, and Fernanda Cássio. 2012. “Effects of Increased Temperature and Aquatic Fungal Diversity on Litter Decomposition.” *Fungal Ecology* 5 (6): 734-40.

Gessner, Mark O. 1997. “Fungal Biomass, Production and Sporulation Associated with Particulate Organic Matter in Streams.” *Limnetica* 13 (2): 33–44.

Gessner, Mark O., and Eric Chauvet. 1997. “Growth and Production of Aquatic Hyphomycetes in Decomposing Leaf Litter.” *Limnology and Oceanography* 42 (3): 496–505.

Gessner, Mark O., Christopher M. Swan, Christian K. Dang, Brendan G. McKie, Richard D. Bardgett, Diana H. Wall, and Stephan Hättenschwiler. 2010. “Diversity Meets Decomposition.” *Trends in Ecology and Evolution* 25. Elsevier Ltd: 372–80.

Ghalambor, Cameron K, Raymond B Huey, Paul R Martin, Joshua J Tewksbury, and George Wang. 2006. “Are Mountain Passes Higher in the Tropics? Janzen’s Hypothesis Revisited.” *Integrative and Comparative Biology* 46 (1): 5–17.

Goering, H.K., and P.J. Van Soest. 1970. *Forage Fiber Analyses (Apparatus,Reagents, Procedures and Some Applications)*. Washington, D.C.

Gonçalves, J. F., Manuel A.S. Graça, and M. Callisto. 2007. “Litter Decomposition in a Cerrado Savannah Stream Is Retarded by Leaf Toughness, Low Dissolved Nutrients and a Low Density of Shredders.” *Freshwater Biology* 52 (8): 1440–51.

Gonçalves, José F, Manuel A.S. Graça, and Marcos Callisto. 2006. “Leaf-Litter Breakdown in 3 Streams in Temperate, Mediterranean and Tropical Cerrado Climates.” *Scoiety of Freshwater Science* 25 (2): 344–55.

Graça, Manuel A.S. and Veronica Ferreira. 2001. “Litter Processing along a Stream Gradient : The Role of Invertebrates and Decomposers Litter Processing along a Stream Gradient : The Role of Invertebrates and Decomposers.” *North American Benthological Society* 20 (3): 408–20.

Graça, Manuel A.S., and J. M. Poquet. 2014. “Do Climate and Soil Influence Phenotypic Variability in Leaf Litter, Microbial Decomposition and Shredder Consumption?” *Oecologia* 174 (3): 1021–32.

Graça, Manuel A.S.. 2001. “The Role of Invertebrates on Leaf Litter Decomposition in Streams – a Review of Organic Matter.” *Hydrobiology* 86: 383–94.

Graça, Manuel A.S., Felix Baerlocher, and Mark O. Gessner. 2005. *Methods to Study Litter Decomposition*. Edited by Manuel A.S. Graça, Felix Bärlocher, and Mark O. Gessner. Berlin/Heidelberg: Springer-Verlag.

Graça, Manuel A.S., and Cristina Canhoto. 2006. “Leaf Litter Processing in Low Order Streams.” *Limnetica* 25 (1-2): 1–10.

Graça, Manuel A.S., and Claudia Cressa. 2010. “Leaf Quality of Some Tropical and Temperate Tree Species as Food Resource for Stream Shredders.” *International Review of Hydrobiology* 95 (1): 27–41.

Graham, Catherine H, Juan L Parra, Carsten Rahbek, and Jimmy a McGuire. 2009. “Phylogenetic Structure in Tropical Hummingbird Communities.” *Proceedings of the National Academy of Sciences of the United States of America* 106 Suppl (November): 19673–78.

Grattan, Robert, and Keller Suberkropp. 2001. “Effects of Nutrient Enrichment on Yellow Poplar Leaf Decomposition and Fungal Activity in Streams.” *Journal of the North American Benthological.* 20 (1): 33–43.

Gulis, V., and K Suberkropp. 2003. “Leaf Litter Decomposition and Microbial Activity in Nutrient-Enriched and Unaltered Reaches of a Headwater Stream.” *Freshwater Biology* 48: 123–34.

Gulis, Vladislav, and Keller Suberkropp. 2003. “Interactions between Stream Fungi and Bacteria Associated with Decomposing Leaf Litter at Different Levels of Nutrient Availability.” *Aquat Microb Ecol* 30 (Bengtsson 1992): 149–57.

Gulis, Vladislav, and Keller Suberkropp. 2004. “Effects of Whole-Stream Nutrient Enrichment on the Concentration and Abundance of Aquatic Hyphomycete Conidia in Transport.” *Mycologia* 96 (1): 57–65.

Hargrave, Barry T. 1972. “Aerobic Decomposition of and Detritus as a Function of Particle Surface Area and Organic Sediment Content.” *Limnology and Oceanography* 17 (4): 583–86.

Hawkins, Bradford a. 2001. “Ecology’s Oldest Pattern?” *Trends in Ecology & Evolution* 16 (8): 470.

Hawkins, Bradford, and José Felizola. 2004. “‘Latitude’ and Geographic Patterns in Species Richness.” *Ecography* 27 (2): 268–72.

Hieber, Maggi, and Mark Gessner. 2002. “Contribution of Stream Detritivores, Fungi, and Bacteria to Leaf Breakdown Based on Biomass Estimates.” *Ecology by the Ecological Society of America* 83 (4): 1026–38.

Hillebrand, Helmut. 2004. “On the Generality of the Latitudinal Diversity Gradient.” *The American Naturalist* 163 (2): 192–211.

Hillebrand, Helmut, and Andrey I Azovsky. 2001. “Body Size Determines the Strength of the Latitudinal Diversity Gradient.” *Ecography* 24 (August 2000): 251–56.

Ho, Wai Hong, Kevin D. Hyde, and I. John Hodgkiss. 2001. “Fungal Communities on Submerged Wood from Streams in Brunei, Hong Kong, and Malaysia.” *Mycological Research* 105 (July 2000): 1492–1501.

Hobbie, Sarah E. 2005. “Contrasting Effects of Substrate and Fertilizer Nitrogen on the Early Stages of Litter Decomposition.” *Ecosystems* 8 (6): 644–56.

Hobbie, Sarah E. 2008. “Nitrogen Effects on Decomposition: A Five-Year Exeriment in Eight Temperate Sites.” *Ecologial Society of America* 89 (9): 2633–44.

Hoiss, Bernhard, Jochen Krauss, Simon G Potts, Stuart Roberts, and Ingolf Steffan-Dewenter. 2012. “Altitude Acts as an Environmental Filter on Phylogenetic Composition, Traits and Diversity in Bee Communities.” *Proceedings. Biological Sciences / The Royal Society* 279 (1746): 4447–56.

Irons, John G, Mark Oswood, Jean Stout, and Cathy M Pringle. 1994. “Latitudinal Pattems in Leaf Litter Breakdown : Is Temperature Really Important ?” *Freshwater Biology* 32: 401–11.

Jabiol, Jérémy, Andreas Bruder, Mark O. Gessner, Marika Makkonen, Brendan G. McKie, Edwin T.H.M. Peeters, Veronique C.a. Vos, and Eric Chauvet. 2013. “Diversity Patterns of Leaf-Associated Aquatic Hyphomycetes along a Broad Latitudinal Gradient.” *Fungal Ecology* 6 (5): 439–48.

Janzen, Daniel. 1967. “Why Mountain Passes Are Higher in the Tropics.” *The American Naturalist* 101 (No.919): 233–49.

Karp, Gerald. 2005. *Biología Celular Y Molecular: Conceptos Y Experimentos*. Edited by Martha Araiza and Ismael Vásquez. Fourth. México: McGraw-Hill Interamericana.

Kaspari, Michael, Milton N. Garcia, Kyle E. Harms, Mirna Santana, S. Joseph Wright, and Joseph B. Yavitt. 2008. “Multiple Nutrients Limit Litterfall and Decomposition in a Tropical Forest.” *Ecology Letters* 11 (1): 35–43.

Kätterer, T., M. Reichstein, O. Andrén, and a. Lomander. 1998. “Temperature Dependence of Organic Matter Decomposition: A Critical Review Using Literature Data Analyzed with Different Models.” *Biology and Fertility of Soils* 27 (3): 258–62.

Kavahagh, Kevin, ed. 2005. “Introduction to Fungal Physiology.” In *Fungi: Biology and Aplications*, 15–28.

Kirschbaum, Miko U.F. 1995. “The Temperature Dependence of Soil Organic Matter Decomposition, and the Effect of Global Warming on Soil Organic C Storage.” *Soil Biology and Biochemistry* 27 (6): 753–60.

Kocher, Scott, and Ernest Williams. 2000. “The Diversity and Abudnance of North American Butterflies Vary with Habitat Disturbance and Geography.” *Journal of Biogeography* 27 (4): 785–94.

Körner, Christian. 2007. “The Use of ‘Altitude’ in Ecological Research.” *Trends in Ecology & Evolution* 22 (11): 569–74.

Krauss, Gerd Joachim, Magali Solé, Gudrun Krauss, Dietmar Schlosser, Dirk Wesenberg, and Felix Bärlocher. 2011. “Fungi in Freshwaters: Ecology, Physiology and Biochemical Potential.” *FEMS Microbiology Reviews* 35 (4): 620–51.

Krebs, Charles. 2001. *Ecology*. 5th ed. San Francisco: Benhamin Cummings.

Lecerf, Antoine, Geta Risnoveanu, Cristina Popescu, Mark Gessner, and Eric Chauvet. 2007. “Decomposition of Diverse Litter Mixtures in Streams.” *Ecology* 88 (1): 219–27.

Lindstrom, E.S. 2000. “Bacterioplankton Community Composition in Five Lakes Differing in Trophic Status and Humic Content.” *Microbial Ecology* 40: 104–13.

Lindström, Eva S, Miranda P Kamst-van Agterveld, Eva S Lindstro, and Gabriel Zwart. 2005. "Distribution of Typical Freshwater Bacterial Groups is Associated with pH , Temperature , and Lake Water Retention Time" *Applied and Environmental Microbiology* 71 (12): 8201–6.

Lomilino, Mark. V. 2001. "Elevational Gradients of Species-Density: Historical and Prospective Views." *Global Ecology and Biogeography* 10: 3–13.

MacArthur, Robert, and Edward Wilson. 1963. "An Equilibrium Theory of Insular Zoogeography." *Iternational Journal of Organic Evolution* 17 (4): 373–87.

Maharning, Ardhini Rin, and Felix Barlocher. 1996. "Growth and Reproduction in Aquatic Hyphomycetes." *Mycological Society of America* 88 (1): 80–88.

Martínez, Aingeru, Aitor Larrañaga, Javier Pérez, Enrique Descals, and Jesús Pozo. 2014. "Temperature Affects Leaf Litter Decomposition in Low-Order Forest Streams: Field and Microcosm Approaches." *FEMS Microbiology Ecology* 87 (1): 257–67.

Martiny, Jennifer B Hughes, Brendan J M Bohannan, James H Brown, Robert K Colwell, Jed a Fuhrman, Jessica L Green, M Claire Horner-Devine, et al. 2006. "Microbial Biogeography: Putting Microorganisms on the Map." *Nature Reviews. Microbiology* 4 (February): 102–12.

Medeiros, a. O., C. Pascoal, and M. a. S. Graça. 2009. "Diversity and Activity of Aquatic Fungi under Low Oxygen Conditions." *Freshwater Biology* 54 (1): 142–49.

Ministerio de Agricultura, Ganadería, Acuacultura y Pesca (MAGAP). 2012. "Mapa de Suelos (variable Taxonomia)." *Reública Del Ecuador*. http://geoportal.magap.gob.ec/mapas/pichincha/mapa_suelos_taxonomia_pichincha.pdf.

Mittelback, Gary, Christopher Steiner, Samuel Scheiner, Katherine Gross, Heather Reynolds, Robert Waide, Michael Willig, Stanley Dodson, and Laura Gough. 2001. "What is the Observed Relationsihp between Species Richness and Productivity?" *Ecology* 82 (9): 2381–96.

Molles, Manuel (University of New Mexico). 2010. "Natural History and Evolution." In *Ecology, Concepts and Applications*, Fifth edit, 10–77. Boston: McGraw-Hill.

Muyzer, G, and K Smalla. 1998. "Application of Denaturing Gradient Gel Electrophoresis (DGGE) and Temperature Gradient Gel Electrophoresis (TGGE) in Microbial Ecology." *Antonie van Leeuwenhoek* 73 (1): 127–41.

Muyzer, Gerard, Ellen C D E Waal, and Andre G Uitierlinden. 1993. "Profiling of Complex Microbial Populations by Denaturing Gradient Gel Electrophoresis Analysis of Polymerase Chain Reaction-Amplified Genes Coding for 16S rRNA." *Applied and Environmental Microbioology* 59 (3): 695–700.

National Aeronautics and Space Administration: NASA. 2014. "Temperature and Altitude." *June 12*. http://www.grc.nasa.gov/WWW/k-12/problems/Jim_Naus/TEMPandALTITUDE_ans.htm.

Pascoal, Cláudia, and Fernanda Cássio. 2004. “Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River Contribution of Fungi and Bacteria to Leaf Litter Decomposition in a Polluted River.” *Applied and Environmental Microbiology* 70 (9): 5266–73.

Perez-Harguindeguy, N, S Diaz, Johannes H. C. Cornelissen, F Vendramini, M Cabido, and a Castellanos. 2000. “Chemistry and Toughness Predict Leaf Litter Decomposition Rates over a Wide Spectrum of Functional Types and Taxa in Central Argentina.” *Plant and Soil* 218: 21–30.

Peterson, Michelle E., Robert Eisenthal, Michael J. Danson, Alastair Spence, and Roy M. Daniel. 2004. “A New Intrinsic Thermal Parameter for Enzymes Reveals True Temperature Optima.” *Journal of Biological Chemistry* 279 (20): 20717–22.

Pianka, Eric R. 1966. “Latitudinal Gradients in Species Diversity: A Review of Concepts.” *The American Naturalist* 100 (910): 33.

Poff, N Leroy, William Funk, Cameron Ghalambor, Boris Kondretieff, Steven Thomas, Alexander Flecker, Joseph Bernardo, and Kelly Zamudio. 2010. “EVOTRAC Project.” Vol. 267.

Powers, Jennifer S., and Stefani Salute. 2011. “Macro- and Micronutrient Effects on Decomposition of Leaf Litter from Two Tropical Tree Species: Inferences from a Short-Term Laboratory Incubation.” *Plant and Soil* 346 (1): 245–57.

Rahbek, Carsten. 2004. “The Role of Spatial Scale and the Perception of Large-Scale Species-Richness Patterns.” *Ecology Letters* 8 (2): 224–39.

Raich, J.W., and W.H. Schlesinger. 1992. “The Global Carbon Dioxide Flux in Soil Respiration and Its Relationship to Vegetation and Climate.” *TELLUS* 44B: 81–99.

Rendón, María José. 2012. “Hifomicetes Tropicales de Páramo y su Rol en la Descomposición de Hojarasca.” Univesidad San Francisco de Quito.

Rex, Michael, Alitair Crame, Carol Sturat, and Andrew Clarke. 2005. “Large-Scale Biogeographic Patterns in Marine Mollusks: A Confluence of History and Productivity?” *Ecology* 86 (9): 2288–97.

Rezende, Renan S, Mauricio M Petrucio, and José F Gonçalves. 2014. “The Effects of Spatial Scale on Breakdown of Leaves in a Tropical Watershed.” *PloS One* 9 (5): e97072.

Rosenzweig, Michael, and Elizabeth A Sandlin. 1997. “Species Diversity and Latitudes: Listening to Area’s Signal.” *Nordic Society Oikos* 80 (May): 172–76.

Rosenzwig, Michael. 1992. “Species Diversity Gradients: We Know More and Less than We Thought.” *American Society of MammalogY* 73 (4): 715–30.

Royer, Todd, and Wayne Minshall. 2003. “Controls on Leaf Processing in Streams from Spatial-Scaling and.” *The North American Benthological Society* 22 (3): 352–58.

Sahadevan, Seena, Sofia Duarte, Cláudia Pascoal, and Fernanda Cássio. 2012. “Intraspecific Variation of the Aquatic Fungus Articulospora Tetracladia: An Ubiquitous Perspective.” *PloS One* 7 (4): e35884.

Sanders, Nathan J. 2002. “Elevational Gradients in Ant Species Richness: Area, Geometry, and Rapoport’s Rule.” *Ecography* 25: 25–32.

Sangkyu, Park, and Cho Kang-Hyun. 2003. “Nutrient Leaching from Leaf Litter of Emergent Macrophyte (Zizania Latifolia) and the Effects of Water Temperature on the Leaching Process.” *Korean Journal of Biological Sciences* 7 (4): 289–94.

Sarmiento, G. 1986. “Ecologically Crucial Features of Climate in High Tropical Mountains.” *High Altitude Tropical Biogeography*, 11–45.

Schindler, Markus, and Mark Gessner. 2009. “Functional Leaf Traits and Biodiversity Effects on Litter Decomposition in a Stream.” *Ecological Society of America* 90 (6): 1641–49.

Schlötterer, Christian. 2004. “The Evolution of Molecular Markers--Just a Matter of Fashion?” *Nature Reviews. Genetics* 5 (January): 63–69.

Schmid, Bernhard. 2002. “The Species Richness–productivity Controversy.” *Trends in Ecology & Evolution* 17 (3): 113–14.

Schneider, Thomas, Bertran Gerrits, Regula Gassmann, Emanuel Schmid, Mark O Gessner, Andreas Richter, Tom Battin, Leo Eberl, and Kathrin Riedel. 2010. “Proteome Analysis of Fungal and Bacterial Involvement in Leaf Litter Decomposition.” *Proteomics* 10 (9): 1819–30.

Shearer, Carol a., Enrique Descals, Brigitte Kohlmeyer, Jan Kohlmeyer, Ludmila Marvanová, David Padgett, David Porter, et al. 2006. “Fungal Biodiversity in Aquatic Habitats.” *Biodiversity and Conservation* 16 (1): 49–67.

Sridhar, K R, and F Bärlocher. 1993. “Effect of Temperature on Growth and Survival of Five Aquatic Hyphomycetes,” 377–87.

Sridhar, K. R., Felix Bärlocher, Gerd Joachim Krauss, and Gudrun Krauss. 2005. “Response of Aquatic Hyphomycete Communities to Changes in Heavy Metal Exposure.” *International Review of Hydrobiology* 90 (1): 21–32.

Stevens, George C. 1992. “The Elevational Gradient in Altitudinal Range : An Extension of Rapoport ’ S Latitudinal Rule to Altitude Author (S): George C . Stevens Reviewed Work (S): Source : The American Naturalist , Vol . 140 , No . 6 (Dec ., 1992), Pp . 893-911 Published B.” *American Society of Naturalists* 140 (6): 893–911.

Suberkropp, Keller, and Eric Chauvet. 1995. “Regulation of Leaf Breakdown by Fungi in Streams : Influences of Water Chemistry.” *Ecological Society of America* 76 (5): 1433–45.

Taylor, Barry R., and Eric E. Chauvet. 2014. “Relative Influence of Shredders and Fungi on Leaf Litter Decomposition along a River Altitudinal Gradient.” *Hydrobiologia* 721 (1): 239–50.

Tjoelker, Mark, Jacek Oleksyn, and Peter Reich. 2001. “Modelling Respiration of Vegetation : Evidence for a General Temperature-Dependent Q 10.” *Global Change Biology* 7: 223–30.

Todd, Royer, and Wayne Minshall. 2001. “Effects of Nutrient Enrichment and Leaf Quality on the Breakdown of Leaves in a Hardwater Stream.” *Freshwater Biology* 46: 603–10.

Vannote, Robin L., G.Wayne Minshall, Kenneth W. Cummins, James R. Sedell, and Colbert E. Cushing. 1980. “The River Continuum Concept.” *Fish.Aquat.Sci.* 37 (River concept): 7.

Waide, R B, M R Willig, C F Steiner, G Mittelbach, L Gough, S I Dodson, G P Juday, and R Parmenter. 1999. “The Relationship between Productivity and Species Richness.” *Annu. Rev. Ecol. Syst.* 30: 257–300.

Waltz, James P. 1969. “Hydrology and Water Quality Studies in the Cache La Poudre Basin, Colorado.” Colorado State University- Fort Collins, Colorado.

White, T.J., T. Bruns, S. Lee, and J. Taylor. 1990. “Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics.” In *PCR Protocols: A Guide to Methods and Applications*, edited by MA Innis, DH Gelfand, JJ Sninsky, and TJ White, 315–22. New York: Academic Press.

Whittaker, R H. 1972. “Evolution and Measurement of Species Diversity.” *International Association for Plant Taxonomy* 21 (2/3): 213–51.

Willig, M.R., D.M. Kaufman, and R.D. Stevens. 2003a. “Latitudinal Gradients of Biodiversity: Pattern, Process, Scale, and Synthesis.” *Annual Review of Ecology, Evolution, and Systematics* 34 (1): 273–309.

Wong, Michelle K M, Teik-khiang Goh, I John Hodgkiss, Kevin D Hyde, V Mala Ranghoo, Clement K M Tsui, Wai-hong Ho, Wilson S W Wong, and Tsz-kit Yuen. 1998. “Role of Fungi in Freshwater Ecosystems.” *Biodiversity and Conservation* 1206: 1187–1206.

Wu, Yongjie, Robert K. Colwell, Carsten Rahbek, Chunlan Zhang, Qing Quan, Changke Wang, and Fumin Lei. 2013. “Explaining the Species Richness of Birds along a Subtropical Elevational Gradient in the Hengduan Mountains.” Edited by K. C. Burns. *Journal of Biogeography* 40 (12): 2310–23.

Wurzbacher, Christian, Janice Kerr, and Hans-Peter Grossart. 1998. “Aquatic Fungi.” In *The Dynamical Processes of Biodiversity- Case Studies of Evolution and Spatial Distribution*, edited by Oscar Grillo, 227–58.

Yamamoto, Nobuyuki, and Glenn Lopez. 1985. “Bacterial Abundance in Relation to Surface Area and Organic Content of Marine Sediments.” *Journal of Experimental Marine Biology and Ecology* 90 (3): 209–20.