Exploring Freshwater Algal Communities in Tropical High-Altitude Lakes and Streams from Southern Ecuador

CARTUCHE PAQUI, Victor Alonso

Abstract
This thesis explored two types of tropical high-altitude freshwater ecosystems located in Southern Ecuador: shallow paramo lakes and mountain streams. Apart from describing some of their basic characteristics for the very first time, we tested three different aspects of the composition and functioning of ecological systems. First, the biodiversity and ecosystem functioning relationship. Second, the distance-decay hypothesis and third, the impact of the degradation of the surrounding vegetation on stream water condition. This thesis provides a baseline for future studies on the impact of climate change and human activities on tropical high-altitude freshwater lakes. These results suggest that by modifying the abiotic and biotic parameters of tropical high-altitude lakes, human activities can indirectly impact their functioning and their capacity to provide vital ecosystem services. No links were found between geographic distance and phytoplankton composition. On the contrary, some environment related patterns of community structure were observed. Regarding streams, the results report the differences in the water condition […]

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Exploring Freshwater Algal Communities in Tropical High-Altitude Lakes and Streams from Southern Ecuador

THÈSE

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La Faculté des sciences, sur le préavis de Monsieur B. W. IBELINGS, professeur ordinaire et directeur de thèse (Département F.-A. Forel des sciences de l'environnement et de l'eau), Monsieur P. VENAIL, professeur et codirecteur de thèse (Universidad de Ingeniería y Tecnología (UTEC), Lima, Peru), Madame K. MANOYLOV, professeure (Department of Biological and Environmental Sciences, Georgia College and State University, Milledgeville, Georgia, USA) et Monsieur A. CAMACHO, professeur (Cavanilles Institute, University of Valencia, Spain), autorise l'impression de la présente thèse, sans exprimer d'opinion sur les propositions qui y sont énoncées.

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Abstract

We are in the middle of multiple major environmental crises, including climate change, biodiversity loss and freshwater scarcity. In this harsh environmental context, freshwater ecosystems are particularly vulnerable. Rivers, streams, lakes and other freshwater systems are habitats for a large diversity of lifeforms and provide important services such as water storage and regulation of the hydrological cycle. Therefore, it is important to better characterize these waterbodies to ensure their preservation and protection. To date, freshwater research has been mostly focused on waterbodies from temperate regions and lowlands. Studies on tropical high-altitude freshwater ecosystems are much less common. For many of these, we still lack information on their basic properties such as morphology, water physics and chemistry. Even less is known about their biotic component. They are also under much pressure due to human activities including mining, agriculture and urbanization. They are also very sensitive to climate change. I focused on microalgae because they are the major primary producers in freshwater ecosystems, representing very often the base of the entire trophic web. Microalgae are responsible for major functions such as nutrient uptake, oxygen production, CO₂ fixation and biomass production. The main purpose of this thesis is to address major gaps in the understanding of tropical high-altitude freshwater ecosystems, which are understudied compared to the freshwater ecosystems from other parts of the world. Filling these gaps would help ensuring a continuation of healthy ecosystem functioning in a time when freshwater ecosystems are under immense anthropogenic pressure.

My thesis work is based on two fieldwork campaigns in Southern Ecuador during November 2016. In the first campaign, I sampled a group of lakes located in a tropical high-altitude wetland called páramo at over 3200 meters above sea level. In the second campaign, I sampled a series of streams located near the city of Loja in Ecuador, over 2100 meters above sea level. The observational studies that I performed embrace the actual complexity and beauty of natural ecosystems and patterns, whilst at the same time this approach makes sacrifices in the level of mechanistic understanding that can be reached. My thesis is built around three key ecological concepts using algae from Andean high-altitude lakes and mountain streams as study systems: 1) biodiversity and ecosystem functioning, 2) the distance-decay hypothesis and 3) the impact of the degradation of the adjacent vegetation on stream water condition, using diatoms as bioindicators.
Chapter I is a general introduction that provides basic information on relevant topics to be addressed in this thesis. It also presents the structure of the thesis and an overview of the content of the different chapters. In Chapter II, I explore and describe for the first time the general characteristics of a group of 24 tropical high-altitude freshwater lakes in southern Ecuador. This chapter may provide a baseline for future studies on the impact of climate change and human activities on tropical high-altitude freshwater lakes. Chapter III deals with biodiversity and ecosystem functioning (BEF) in tropical high-altitude lakes. There is a total absence of BEF studies from high altitude tropical regions. I explored the links between several environmental variables and productivity, measured as chlorophyll-a concentration and total phytoplankton biovolume. I found that a combination of four abiotic factors explained over three quarters of the variation in chlorophyll-a concentration amongst lakes. Taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolume. Moreover, Shannon’s diversity index was negatively correlated to both chlorophyll-a concentrations and total phytoplankton biovolume, presumable due to a strong compositional effect. These results suggest that by modifying the abiotic and biotic parameters of tropical high-altitude lakes, human activities can indirectly impact their functioning and their capacity to provide vital ecosystem services. In Chapter IV, I tested the geographical and environmental components of the distance decay relationships in the phytoplankton structure across tropical high-altitude lakes. Phytoplankton composition at the phyla level showed high among-lake variation in the tropical high-altitude lakes from Tres Lagunas. I found no links between the geographic distance and phytoplankton composition. On the contrary, I observed some environment related patterns of community structure. Despite not proving a mechanistic explanation for the observed patterns of community structure, my findings will help improving our comprehension on these vulnerable and vital ecosystems. Chapter V reports the impacts of the degradation of the surrounding vegetation in the periphyton composition of tropical mountain streams with different levels of degradation in their watersheds. I depicted differences in the water condition according to the type of vegetation in the watershed. Moreover, the diatom communities also responded to changes in land use in the watersheds. Diatom communities from stream zones with the natural vegetation cover were as different from each other as communities from different streams. Finally, Chapter VI offers a brief general discussion and recapitulates the major findings of the different chapters of this thesis. It
show how all chapters fit together and contribute to the field and put the different parts of the thesis into a wider perspective.

Résumé

Nous traversons actuellement multiples crises environnementales majeures, notamment le changement climatique, la perte de biodiversité et la rareté de l'eau douce. Dans ce contexte environnemental difficile, les écosystèmes d'eau douce sont particulièrement vulnérables. Les rivières, ruisseaux, lacs et autres systèmes d'eau douce offrent des habitats pour une grande diversité de formes de vie et fournissent des services importants tels que le stockage de l'eau et la régulation du cycle hydrologique. Par conséquent, il est important de mieux caractériser ces corps d'eau pour assurer leur conservation et leur protection. À ce jour, la recherche sur l'eau douce s'est principalement concentrée sur les plans d'eau des régions tempérées et des basses terres. Les études sur les écosystèmes d'eau douce tropicaux de haute altitude sont plus rares. Pour beaucoup d'entre eux, nous manquons encore d'informations sur leurs propriétés de base telles que la morphologie, la physique et la chimie de l'eau. On en sait encore moins sur leur composante biotique. Ils subissent également de fortes pressions en raison des activités humaines, notamment l'exploitation minière, l'agriculture et l'urbanisation. Ils sont également très sensibles au changement climatique. Dans ma thèse, je me suis concentré sur les microalgues car ce sont les principaux producteurs primaires dans les écosystèmes d'eau douce, représentant très souvent la base de l'ensemble du réseau trophique. Les algues sont responsables de fonctions importantes telles que l'absorption des nutriments, la production d'oxygène, la fixation du CO2 et la production de biomasse. Le principal objectif de cette thèse est de combler les lacunes dans la compréhension des écosystèmes d'eau douce tropicaux de haute altitude, qui sont sous-étudiés par rapport aux écosystèmes d'eau douce d'autres parties du monde. Ceci aidera à assurer le maintien d'un fonctionnement sain de l'écosystème à une époque où les écosystèmes d'eau douce sont soumis à une immense pression anthropique.

Mon travail de thèse est basé sur deux campagnes de terrain dans le sud de l'Équateur en novembre 2016. Dans la première campagne, j'ai échantillonné un groupe de lacs situés dans une zone humide tropicale de haute altitude appelée páramo à plus de 3200 mètres d'altitude. Dans la deuxième campagne, j'ai échantillonné une série de cours d'eau situés...
près de la ville de Loja en Équateur, à plus de 2100 mètres d'altitude. Les études d'observation que j'ai effectuées embrassent la complexité et la beauté réelles des écosystèmes et des modèles naturels, tandis que cette approche fait en même temps des sacrifices dans le niveau de compréhension mécaniste qui peut être atteint. Ma thèse s'articule autour de trois concepts écologiques clés utilisant les algues des lacs andins de haute altitude et des ruisseaux de montagne comme systèmes d’étude : 1) la biodiversité et le fonctionnement des écosystèmes, 2) l'hypothèse de « distance-decay » 3) l'impact de la dégradation de la végétation adjacente sur l'état de l'eau du ruisseau, en utilisant des diatomées comme bioindicateurs.

Le chapitre I est une introduction générale qui fournit des informations de base sur des sujets pertinents à traiter dans cette thèse. Il présente également la structure de la thèse et un aperçu du contenu des différents chapitres. Dans le chapitre II, j’explore et décrit pour la première fois les caractéristiques générales d'un groupe de 24 lacs d'eau douce tropicaux de haute altitude dans le sud de l'Équateur. Ce chapitre fournit une base de référence pour de futures études sur l'impact du changement climatique et des activités humaines sur les lacs d'eau douce tropicaux de haute altitude. Le chapitre III traite de la biodiversité et du fonctionnement des écosystèmes (BEF) dans les lacs tropicaux de haute altitude. Il y a une absence totale d'études BEF dans les régions tropicales de haute altitude. J'ai exploré les liens entre plusieurs variables environnementales et la productivité, mesurées en tant que concentration de chlorophylle-a et biovolume de phytoplancton total. J'ai trouvé qu'une combinaison de quatre facteurs abiotiques expliquait plus des trois quarts de la variation de la concentration de chlorophylle-a entre les lacs. La richesse des taxons n'était liée ni aux concentrations de chlorophylle-a ni au biovolume total de phytoplankton. De plus, l’indice de diversité de Shannon était corrélé négativement aux concentrations de chlorophylle-a et au biovolume total de phytoplankton, vraisemblablement en raison d’un fort effet de composition. Les résultats suggèrent qu’en modifiant les paramètres abiotiques et biotiques des lacs tropicaux de haute altitude, les activités humaines peuvent indirectement affecter leur fonctionnement et leur capacité à fournir des services écosystémiques vitaux. Dans le chapitre IV, j’ai testé les composantes géographiques et environnementales des relations « distance-decay » dans la structure du phytoplancton à travers les lacs tropicaux de haute altitude. La composition du phytoplancton au niveau des phyla a montré une forte variation entre les lacs dans les lacs tropicaux de haute altitude de Tres Lagunas. Je n'ai trouvé aucun
lien entre la distance géographique et la composition du phytoplancton. Au contraire, j'ai observé certains modèles de structure communautaire liés à l'environnement. Bien que cela ne prouve pas une explication mécaniste des modèles de structure communautaire observés, mes résultats aideront à améliorer notre compréhension de ces écosystèmes vulnérables et vitaux. Le chapitre V rapporte les impacts de la dégradation de la végétation environnante sur la composition du périphyton des ruisseaux de montagne tropicaux avec différents niveaux de dégradation dans leurs bassins versants. J'ai décrit les différences dans l'état de l'eau selon le type de végétation dans le bassin versant. De plus, les communautés de diatomées ont également réagi aux changements d'utilisation des terres dans les bassins versants. Les communautés de diatomées des zones de cours d'eau avec différents couvert végétaux étaient aussi différentes les unes des autres que les communautés de différents cours d'eau. Enfin, le chapitre VI propose une brève discussion générale et récapitule les principales conclusions des différents chapitres de ma thèse. Il montre comment tous les chapitres s'emboîtent et contribuent au domaine et mettent les différentes parties de cette thèse dans une perspective plus large.
CHAPTER I

General Introduction

The purpose of this general introduction is to provide readers with basic information on the major topics to be addressed in this thesis. It also helps presenting the structure of the thesis and the content of the different chapters.

Freshwater ecosystems

Major freshwater ecosystems include rivers, streams, lakes, reservoirs, wetlands and ponds. They are ecologically very important because they provide habitat for an impressive diversity of organisms, from bacteria to large mammals. They also play a major role in the hydrological cycle by circulating freshwater from high-altitude terrestrial ecosystems to the ocean, they provide water for irrigation, power generation and industrial purposes. Moreover, some of them contribute largely to carbon sequestration. Freshwater ecosystems are very sensitive to climate change, especially to temperature increase that affects their thermal structure. It is also well known that ice cover in the mountain glaciers is decreasing, perturbating the freshwater cycle. Freshwater ecosystems are one of the most diverse but are also one of the most threatened by pollution, biodiversity loss and climate change [1]. Lakes are important natural freshwater reservoirs all over the world. Investigating their structure and dynamics is needed to understand how the environmental factors influence their different biotic compartments. The three main limiting factors on the productivity of freshwater ecosystems are light, CO$_2$ and nutrients. Solar radiation is the major energy source in freshwater ecosystems. Photosynthetic organisms such as microscopic algae transform the solar energy and inorganic material into organic matter, allowing other organisms to live. The solar energy can be a limiting factor in aquatic ecosystem. Of the solar radiation entering the water column, a big portion is scattered. The rest of the energy is absorbed by the water and other compounds present in the water, resulting in a decrease of radiation with depth. Dissolved carbon dioxide (CO$_2$) is the main source of carbon for photosynthesis, but the carbon cycle in water is very complex. Carbon dioxide dissolves easily in water and is rapidly converted into carbonic acid. At the same time, carbonic acid dissociates fast, leading to changes in the pH of water. Ultimately, the relative
proportions of carbon dioxide, carbonate and bicarbonate determine the pH of water. Nitrogen and phosphorus are the two main limiting nutrients for phytoplankton. Nitrogen is present as organic compounds including amino acids and as inorganic reactive nitrogen such as ammonia, nitrite (NO$_2$-) and nitrate (NO$_3$-). Phosphate is mostly present in water as organic phosphates in living cells or dead organic material. The most vital inorganic form of phosphate is orthophosphate. Two opposing perspectives on the relative role of nitrogen or phosphorus as the major limiting resource for the productivity of freshwater ecosystems have existed for decades.

**Microalgae**

Microalgae consists of a diverse assemblage of taxonomic groups. The presence of photosynthetic pigments is the primary characteristic of microalgae. These pigments include the chlorophylls, carotenoids, and phycobilins. Chlorophyll-a is the most common chlorophyll and is present in all algae and cyanobacteria. Besides chlorophyll-a, there are chlorophyll-b, c and d than are present in some special groups of algae. The cyanobacteria, also called blue-green algae, are bacteria with photosynthetic pigments. As prokaryotic organisms, they lack cell membranous structures and the cytoplasm contains the proteins and nucleic acids. Cyanobacteria can be unicellular or colonial. Green algae are another group of phytoplankton. They are eukaryotic and extremely morphologically diverse in lakes. Besides the green algae, other algae abundant in freshwater lakes are the Xanthophyceae (yellow-green algae), Chrysophyceae (golden-brown algae). Diatoms are another group of phytoplankton with silicified cell walls. Diatoms are unicellular or colonial as well. The unique silicified cell wall gives diatoms beautiful and complex structures. Additionally, their relatively dense cell wall causes them to sink easier compared to green algae and cyanobacteria. They are mostly non-motile. The fact that there are so many kinds of phytoplankton in different shapes and sizes, with different or similar characters living in lakes draw scientists’ attention. An outstanding feature of phytoplankton communities is the coexistence of numbers of algal species. Early in the 60’s, [2] reported the paradox of plankton, wondering how it is possible for many species to coexist in a relative isotropic or unstructured environment with all competing for the same limited array of resources. This contradicts the competitive exclusion principle, suggesting that there can be no more species coexisting
than limiting resources. This special feature of phytoplankton communities makes them a popular tool for community ecology studies.

**Tropical high-altitude freshwater ecosystems: lakes**

High-altitude lakes provide important hydrological services in Andean regions, such as freshwater for human consumption, irrigation and hydropower production purposes [3,4]. Like high-altitude temperate lakes, tropical high-altitude lakes (TRHALs) have low average water temperatures but extreme diel water temperature variations. They are submitted to strong winds, receive intense solar UV radiations and are generally low in nutrients [5,6]. Because of their tropical location, TRHALs have no seasonality and no ice cover. They present polymictic mixing regimes with often a complex thermal structure, receive intense UV radiation through the year, have high dissolved organic carbon and low UV transparency [4,7-13]. Thus, TRHALs are extreme freshwater ecosystems. One type of TRHALs are the páramo lakes, located above the tree line between approximately 3200 to 4500 meters above sea level (m.a.s.l.) and filled almost exclusively by rain and groundwater. Another type are the glacial lakes, located at higher altitudes and being fed by glacier melting waters. These two types of lakes can show important differences in both abiotic and biotic characteristics [8]. In this thesis, we focus in páramo lakes located between 3288 and 3362 meters above sea level without a direct connection to glaciers. TRHALs also show a wide range of phytoplankton productivity levels, with chlorophyll-a concentrations ranging from below 1 µg l\(^{-1}\) to values over 8 mg l\(^{-1}\) [5,13-17]. Productivity in TRHALs is often nutrient limited [5,13]. Their phytoplanktonic productivity has been negatively related to depth [13], UV radiation [18] and positively to pH [14], total phosphate [13] and total nitrogen [8]. Studies of phytoplankton diversity in TRHALs reported over a hundred different genera in different regions of Ecuador [8,13,17]. Regarding the phytoplankton diversity per lake, previous studies counted from 5 to 45 genera per lake in Southern Ecuador [8,13], with taxonomic richness decreasing with altitude and increasing with conductivity [8]. Despite their major ecological role as sustainers for a rich biota and as providers of hydrological services, human activities are putting TRHALs at risk [3,4,13,19,20] and we still know little about how the abiotic or biotic factors influencing their functioning. This thesis aims to help to fill this gap.
Tropical high-altitude freshwater ecosystems: streams

Andean rivers and streams play a major ecological role providing habitat and water for a huge diversity of life forms, from large mammals to microscopic bacteria. They also provide important hydrological ecosystem services such as freshwater for consumption, irrigation, electricity generation, flood and drought protection [21]. Unfortunately, phenomena like climate change and human driven activities such as agriculture, livestock, hydroelectricity projects and mining and are causing their degradation, directly reducing their biodiversity and water quality [22]. Rivers are the most abundant freshwater ecosystems in Ecuador, especially in Andean and Amazonian regions. In Ecuador, total average runoff is 432,000 hm$^3$/year and that the per-capita water availability is 43,500 m$^3$/habitant/year. This is four times the world average (10,800 m$^3$) and much higher than the European average (2,700 m$^3$, [23]). A study carried out in 123 rivers from high Andean basins in Ecuador and Peru showed a high heterogeneity of rivers in their physiochemical and hydro-morphological parameters [24]. Five environmental factors interact in time and space to determine the structure and functioning of montane streams: 1) water flow, 2) sediment and organic matter input, 3) temperature and light, 4) chemical and nutritional conditions and 5) the connection of plants and animals that influence the rates of ecosystem processes and community structure [25]. Dissolved oxygen values were low in organically contaminated areas and generally pH values were close to neutral or slightly alkaline. Nitrite and nitrate values were low: 0-0.23 mg l$^{-1}$ and 0-3.30 mg l$^{-1}$ respectively, while phosphate values ranged from 0 to 4.06 mg l$^{-1}$. The number studies in Ecuadorian lotic systems is low and the associated biodiversity is poorly described [26]. To date, most ecological studies on river ecosystems and water quality have been based on physical-chemical variables and some have used macroinvertebrate communities. Community structure studies on primary producer microorganism’s and their interactions are scarce in Ecuador [21]. Diatoms are particularly important for studying the condition of lotic systems because they respond quickly to changes in water quality (e.g., pollution), which makes them suitable as bio-indicators to determine the ecological water quality [27,28].
Andean wetlands: los páramos

Ecuador is an extremely biodiverse country, with up to 91 different continental biomes [29]. One of them is the páramos, a neotropical high-altitude wetland ecosystem that covers the highest region of the Northern Andes. It is distributed along the Andes mountains in Peru, Ecuador, Colombia, Venezuela and some can also be found in Costa Rica and Panamá [3]. Páramos provide important hydrological services including water storage and provisioning for over a hundred million people [3,30]. This biome is located between the forest upper limit at approximately 3000 meters to perpetual snow at approximately 5000 meters [31]. The Northern Andes cover a total of 490000 km$^2$ and the páramo occupies an area of approximately 46000 km$^2$ [32]. Ecuador has the largest area of páramo (39.8%), followed by Colombia (30.5%), Peru (24, 6%) and Venezuela (5.2%). Although they occupy a relatively small area, the páramos harbor great biodiversity and are vital for human settlements [3,33]. In Ecuador, there are over 3000 freshwater lakes located between 3200 and 4500 meters above sea level with an average water temperature around 8°C that remains stable all year around [6]. Precipitations can vary between 600 mm for dry páramos and more than 4000 mm in wet paramos [34]. Other characteristics of the páramos are the high organic matter content of soils, which have a significant influence on water regulation and water catchment from rain and accumulated humidity (mist). The soil acts like a sponge absorbing and storing water and then releasing it to the subsoil and surface forming lakes, springs and rivers [3]. Due to their elevated organic acid concentrations, páramos soils are generally acidic with pH ranging between 5 to 7 [35]. The soils are normally very black and damp due to the cold climate, high humidity and in some cases to their volcanic origin. Organic matter decomposition is very slow. These characteristics reflect the fact the páramo is an ecosystem with high rates of carbon fixation. For instance, in the páramo of the Carchi province (Northern Ecuador) soils can store around 1700 ton/C/ha. As a reference, the rainforest soil only stores 50 ton C/ha [36]. The páramo grass (pajonal) can fix up to 20 t/C/ha [37]. The water flowing downwards from the páramos, along the mountain streams is used for irrigation, drinking, sanitary purposes and recreation. In some cases, water is also used for hydroelectric power generation [38]. Although páramos provide key ecosystem services, such as carbon storage, regulation of water flow and water supply for consumption and irrigation, they remain largely unexplored and their physical and chemical characteristics as well as the phytoplankton communities and other ecological
features are poorly described. In addition, the páramos are culturally and religiously very important for local human settlements. Local communities just begun to be included in the preparation of management plans or implementation of conservation projects.

**Biodiversity and ecosystem functioning (BEF)**

Biodiversity (contraction of biological diversity) refers to the variety of living forms present in our planet across several scales of organization, from genes to biomes. Ecosystem (contraction of ecological system) describes an assemblage of interacting organisms living together and connected via fluxes of energy and matter. An ecosystem function is any kind of function performed by one or by an assemblage of living organism, including for instance respiration, primary productivity and nutrient cycling [39]. BEF it is the result of the effects of combined activities of plants, animals, and microorganisms on the physical and chemical conditions of their environment, that is, BEF results from the interactions between and within the different biota levels [40]. Given the incredibly fast erosion of biodiversity over the last few decades [41], scientists are concerned about understanding the nature and magnitude of the implications [42]. This awareness amongst scientists generated in the 90’s a research field called biodiversity-ecosystem functioning (BEF). This field focuses on understanding the consequences of losing biodiversity on the functioning of ecological systems. A key to understand and to predict the ecological consequences of diversity loss is to study the relationship between biodiversity and ecosystem functioning [43,44]. It is now well established that biodiversity often influences ecosystem functioning, with consistent evidence supporting that a reduction in biodiversity negatively affects functioning [45]. However, this general conclusion comes from studies that are mostly simplified experiments with terrestrial plants in which the number of species is manipulated one or a few ecosystem functions are measured afterwards. The direction and shape of the relationship between richness and a function is established and interpreted accordingly. Mechanistically speaking, two main processes have been proposed to explain biodiversity’s influence on community functioning, the sampling and the complementarity effect [45]. The sampling effect suggests that some living forms make a disproportionate contribution to the function. When an assemblage or a set of organisms is dominated by one with low functioning level, this results in low community functioning (called a negative selection effect). On the contrary, when the assemblage is dominated by one organism with a high functioning level, this results into
high functioning (called a positive selection effect). Thus, it is the level of functioning of the dominant organism in an assemblage that will determine the nature of the selection effect (negative or positive). Increasing the variety of organisms in an assemblage increases the chances of including a dominant one. When no dominant organism makes a disproportionate contribution to functioning or when positive and negative selection effects cancel each other, the overall selection effect is null. Thus, regarding the sampling effect, community functioning is not dependent on diversity per se but on the probability of the presence of a dominant organism with a disproportionate contribution to community functioning. The complementarity effect results from interactions among organisms and how they influence functioning. Living forms can have negative interactions (e.g., competition) and interfere with each other’s functioning, resulting in communities functioning less well than expected based on the constitutive organisms (called a negative complementarity effect). Living organisms can also have positive interactions (e.g., facilitation), in which some benefit from the presence of other. This leads to communities performing better than the constitutive monocultures (called positive complementarity effect). In the absence of interactions amongst organisms influencing functioning (called neutral interactions), functioning is simply the accumulation of individual performances (null complementarity effect). In the end, it is the balance between the relative contributions of sampling and complementarity effects that determines the shape of the relationship between diversity and functioning. The importance of diversity as a main driver of ecosystem functioning in real-world conditions is an open debated. Some consider that several abiotic factors may dominate over biodiversity effects on ecosystem functioning in natural conditions. However, a recent meta-analysis shows that after controlling for environmental variables, species richness effects on biomass are stronger in nature than in the laboratory [46].

Distance-decay hypothesis

Community ecologists have generated numerous hypothesis regarding the distribution and coexistence of species within and amongst habitats [47,48]. In this context, the distance decay relationship suggests that similarity amongst communities decreases with geographical distance [49,50]. Geographically distant communities are expected to be more dissimilar in their structure and composition than closer ones. That pattern can be linked to at least two non-exclusive mechanisms. First, communities become less similar
with distance because the environmental conditions become less similar. This assumes a strong correlation between geographic distance and environmental dissimilarity. In community ecology this niche-based process is also called “species sorting” [51]. Second, the dispersal capacity of living organism is limited. This means that even if the environment does not change over space, the limited capacity of an organism to disperse does not allow it to succeed everywhere. Thus, an organism has major chances to be found closer to its original localization rather than far from it [52]. In opposition to species-sorting, this second mechanism considers that organisms perform equally in any environmental conditions (e.g., neutrality). Both forces can contribute to generate a distance decay pattern in community composition [53]. This means that a distance decay pattern alone cannot be used as evidence of the underlying mechanisms. Indeed, actual changes in community structure over space and distance result from a balance between local niche-based processes and regional dispersal capabilities [54]. For microorganism, a niche-based perspective called the Baas-Becking’s hypothesis dominated the literature for decades [55,56]. It suggests that given their small size microorganisms are ubiquitous, but the local environment determines where they can persist. This means that only the capacity of a microorganism to cope with the different local environmental conditions, not its dispersal capabilities, would determine its spatial distribution patterns. Consequently, distance decay relationships in microorganisms may result from a strong correlation between geographic and environmental distance. Species sorting would be the dominant underlying mechanism. However, evidence collected over the last two decades suggests that microorganisms show biogeographical patterns, with both local (e.g., species sorting) and regional (e.g., dispersal) processes being important [57-62]. As for macroorganisms, the distance decay relationships in microorganisms is jointly influenced by local environmental filters and dispersal [50,63,64]. In microalgae, the evidence for local and regional processes dominating community structure is mixed [50,59,63-67]. Regarding the relationship between geographical distances and community similarity in microalgae, some studies found evidence of distance decay relationships [50,63,67] but some others did not [64]. Such discrepancies can be explained because factors such as the spatial scale or the environmental context influence the distance decay relationship in microalgae [65,66]. Moreover, studies also offer mixed evidence on the impact of environmental distance on community structure similarity in freshwater phytoplankton. Some studies support the relationship [63,65,66] but some others do not [50].
**Land use effects on streams**

A key aspect in stream ecology is the study of the impact of the surrounding vegetation on water condition. It regulates temperature, enhances water oxygenation, provides organic material, improves nutrient retention and prevents erosion of stream banks amongst others many benefits [27,68-70]. Big differences in functioning between rivers with and without riparian vegetation have been described [71]. The presence of a healthy surrounding vegetation also offers habitats for different kinds of animals (macroinvertebrates, fish) and plants (periphyton, aquatic plants). Riparian vegetation also acts as a natural filter, preventing large loads of nutrients from agricultural and livestock activities to reach the rivers and to contaminate the water [71]. Human activities in the terrestrial ecosystems surrounding streams put at risk their ecological integrity and functioning capabilities by degraded or destroyed stream habitats and reducing water quality [72]. Much of our knowledge about the impact of land use on montane streams comes from extrapolations from temperate regions. In the tropics, studies on the impact of land use on the biotic compartment of streams have mostly been focused on macroinvertebrates [73-75]. It has been shown that the effect of land use on tropical montane streams is weak, especially around urban areas in Ecuador. Invertebrate litter processing was slower in streams surrounded by pastures rather than forests, whereas other processes were unaffected by changes in the surrounding land cover in montane streams in north-western Ecuador [74]. Studies on the impact of land use degradation on water condition using other biotic compartments in tropical montane ecosystems are rare.

**Diatoms as bioindicators of water condition**

Diatoms play a major role in the biogeochemical cycles and are the trophic foundation of several aquatic ecosystems. They are abundant in almost all aquatic and are the ubiquitous group of microalgae [76]. They play a fundamental function in energy networks in the oceans and according to many studies, they are responsible for 35-40% of primary production in marine ecosystems and 25% of the biosphere's total organic carbon fixation [77]. They are the more speciose group of eukaryotic microalgae and are valuable indicators of water condition [78]. They dominate the periphyton and due to their fast growth, they are the main responsible for the primary productivity in shallow rivers [79]. Diatoms are mainly unicellular, although sometimes can form colonies and filaments.
They are eukaryotic microorganisms, composed 60% of silica (SiO$_2$) which forms a shell called frustule that gives it great hardness and strength [81]. They are divided into two large groups according to their valve symmetry, centric (radially symmetrical) and pennate (bilaterally symmetrical). Some authors suggest that most diatom taxa are still to be discovered and described [82,83]. Benthic diatoms have been largely ignored by ecologists due to difficulties with sampling [84]. However, great progress in this field has been achieved recently [85,86]. Diatoms are highly sensitive to changes in the chemical nature of aquatic ecosystems, especially to salinity, acidity and the concentration of nutrients such as phosphorous, nitrogen, silica and to the presence of contaminants [81]. Thus, diatoms can be used as useful and reliable bioindicators [87-89]. Diatoms can also reflect long-term changes in water chemistry [86,89]. When water condition is altered by changes in the water conditions, sudden shifts in diatom community composition help depicting changes in the environment [90]. Some water quality monitoring methods in rivers and streams are based in evaluating changes in the composition of benthic diatoms [91,92]. In some well-studied species, their ranges of environmental conditions and tolerances are known [93]. Therefore, the evaluation of changes in the diatom community composition can provide precise information about environmental changes that occur in a river [94].

Achievements from members of my research group in phytoplankton community ecology related to my thesis

Before and during my thesis, Dr. Patrick Venail co-supervisor and member of my research group had important achievements in BEF studies with phytoplankton as model system. He mostly focused on testing basic ecological hypotheses on community assembly and the relationship between biodiversity and ecosystem functioning (BEF). He’s been working for the last eight years with microscopic algae to address BEF related issues [95-98]. His research in the field of biodiversity and ecosystem functioning using freshwater phytoplankton as model system can be divided into three topics that are briefly described below.
Phylogenetic diversity does not predict community biomass stability nor the nature and strength of species interactions in experimental phytoplankton communities.

Dr. Venail and his colleagues experimentally explored the influence of the evolutionary relatedness of freshwater green algae on the temporal stability of community biomass production [99], and the nature and strength of species interactions [97]. In both cases, they performed laboratory experiments in which manipulated the phylogenetic distance between freshwater green algae species. In [99], they explored how the different components of community temporal stability in the face of environmental fluctuations changed as species got less related. They found that species interactions were more important for community stability than the differences among individual species in their responses to environmental fluctuations. In [97], they first reviewed the empirical evidence linking evolutionary relatedness to the nature and strength of species interactions and found that most of studies performed up to date offered no evidence that relatedness influences species interactions. Then, they tested the hypothesis suggesting that closely related species compete stronger than distantly related species through an experimental approach. They found that neither the nature nor the strength of species interactions among freshwater algae was determined by their evolutionary relatedness.

Evolutionary relatedness does not predict competition or species co-occurrence in natural and experimental communities of green algae.

To explore more natural systems, Dr. Venail and colleagues included transcriptomic analysis intended to identify the genes responsible for species coexistence and the production of biomass in both experimental and natural freshwater green algae [95]. For this, they developed molecular phylogenetics to answer specific questions about the evolution of green algae, to characterize the process of algal diversification and to evaluate the impact of their diversity on ecosystem functioning. They found that evolutionary relatedness did not predict competition or species co-occurrence in natural or experimental communities of green algae.
Niche differences trump fitness differences in predicting phytoplankton coexistence in size differences-based invasion experiments.

The modern framework of coexistence, suggesting a balance between stabilizing and equalizing forces for understanding the maintenance of diversity, may benefit from the incorporation of trait information. In this study, Dr. Venail and colleagues [100] focused on size, a key trait known for capturing several of the physiological and ecological functions of phytoplankton [101] but whose influence at the community level remains largely unexplored. They tested if size differences among cyanobacteria species could determine their coexistence by analyzing the influence of size variability on interspecific niche and relative fitness differences and their relative contribution to the outcome of competition. Coexistence of pairwise combinations of freshwater cyanobacterial species was experimentally tested using an invasion-from-rare approach under controlled laboratory microcosms. Their study included thirty unique pairs of residents vs. invading cyanobacteria, whose average diameter ranged over two orders of magnitude. They found that differences in size among competing species directly influenced both niche and fitness inequalities, but that species coexistence was mainly driven by the niche differences based on such size differences.

Outline of the thesis

My thesis work is based on two fieldwork campaigns in Southern Ecuador during November 2016. In the first one, we sampled a group of lakes located in a tropical high-altitude wetland called páramo over 3200 meters above sea level. In the second campaign, we sampled a series of streams located near the city of Loja in Ecuador. The seven streams are 2100 meters above sea level. We decided to work on tropical high-altitude freshwater ecosystems because they comprise an enormous biodiversity, but we know little about their characteristics and functioning conditions despite their importance in the water cycle. They provide a plethora of hydrological services but are also under much pressure due to human activities including mining, agriculture and urbanization. They are also very sensitive to climate change.
This thesis focuses on three key ecological concepts using algae from Andean high-altitude lakes and mountain streams as study systems: 1) biodiversity and ecosystem functioning, 2) the distance-decay hypothesis and 3) the impact of the degradation of the surrounding vegetation on stream water condition. We hope the findings of this thesis will help to better understand the structure and functioning of tropical montane freshwater ecosystems. As previously mentioned, much freshwater research has been focused on temperate ecosystems, and we know less well how tropical freshwater ecosystems function. In this thesis, we focused on algae because they are the primary producers in freshwater ecosystems, representing the base of the entire trophic web. Algae are responsible for major functions such as nutrient uptake, oxygen production, CO₂ fixation and biomass production. This thesis proposes a series of fieldwork studies that would provide elements to fill current gaps in our understanding of tropical montane ecosystems.

Chapters II, III and IV address different questions related to the characteristics, functioning and community structure of tropical high-altitude lakes from the Tres Lagunas system in southern Ecuador. Chapter V studies a group of mountain streams located around the City of Loja in Ecuador. These streams show different levels of degradation by human related activities, especially in their watersheds. Two of these streams provide freshwater for human consumption to over 240,000 people.

**In chapter II**, we explored and described for the first time the general characteristics of a group of 24 tropical high-altitude freshwater lakes in southern Ecuador (Saraguro/Yacuambi) located on a páramo at an average altitude of 3,300 meters above sea level. The páramo is a wetland ecosystem of high-altitude mountains in the northern Andes (Peru, Ecuador, Colombia, Venezuela) and Central America, located over 3000 meters above sea level. They are strategic ecosystems particularly affected by climate change and human interventions. Activities such as agriculture, mining, livestock and aquaculture are increasing in these regions, putting at risk the overall condition of the Andean terrestrial and aquatic ecosystems. Tropical high-altitude lakes of glacial or volcanic origin are common in the páramos and provide key ecosystem services, such as water storage, regulation of the water cycle and provisioning of freshwater for consumption and irrigation. Despite their importance, tropical high-altitude lakes are still largely unexplored. Despite an increasing interest over the last few years, their environmental characteristics and their biotic composition are poorly documented. This study aims to characterize the physical, chemical, trophic and phytoplanktonic
characteristics of these lakes. We hope this study will provide a baseline for future studies on the impact of climate change and human activities on tropical high-altitude freshwater lakes. This chapter is in preparation for the journal *Water.*

**Chapter III** deals with biodiversity and ecosystem functioning (BEF) in tropical high-altitude lakes. There is a total absence of BEF studies from high altitude tropical regions. Tropical high-altitude lakes are vital freshwater reservoirs in the Andes, heavily threatened by human activates that may alter their functioning and hamper the provisioning of key ecosystem services such as water supply. Despite their ecological and social relevance, we know little about these waterbodies, especially regarding the factors influencing their functioning. Here, we explored the links between several environmental variables and productivity, measured as chlorophyll-a concentration and total phytoplankton biovolume, across twenty-four tropical high-altitude lakes located over three-thousand meter above sea level in Southern Ecuador. We found that a combination of four abiotic factors explained over three quarters of the variation in chlorophyll-a concentration amongst lakes. Contrary to what studies from temperate regions suggest, taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolume. Moreover, Shannon’s diversity index was negatively correlated to both chlorophyll-a concentrations and total phytoplankton biovolume, presumable due to a strong compositional effect. Our results suggest that by modifying the abiotic and biotic parameters of tropical high-altitude lakes, human activates can indirectly affect their functioning and their capacity to provide vital ecosystem services. This chapter is published in the journal *Sustainability.*

**In Chapter IV** we explore the distance decay hypothesis in tropical high-altitude lakes. Describing the patterns and processes of community composition in these lakes is required to better understand the consequences of their degradation by human activities. In this study, we tested the geographical and environmental components of the distance decay relationships in the phytoplankton structure across 24 tropical high-altitude lakes from Southern Ecuador. Phytoplankton composition at the phyla level showed high among-lake variation in the tropical high-altitude lakes from Tres Lagunas. We found no links between the geographic distance and phytoplankton composition. On the contrary, we observed some environmental related patterns of community structure. The absence of support for the distance decay relationship observed here can result from a conjunction
of local niche-based effects and dispersal limitations. Phytoplankton community composition in the Tres Lagunas system or any other ecosystem may be jointly regulated by niche-based and neutral forces that still need to be explored. Despite not proving a mechanistic explanation for the observed patterns of community structure, we hope our findings will help improving our comprehension on these vulnerable and vital ecosystems. More studies in tropical high-altitude lakes are urgently required. This chapter will soon be ready to be submitted to a specialized journal in freshwater ecology.

**Chapter V** reports the impacts of the degradation of the surrounding vegetation in the periphyton composition of tropical mountain streams. The vegetation along a stream helps regulating important processes such as the flow of sediments and nutrients. It also helps stabilizing the riverbanks and regulates the watershed microclimate that ultimately influences the primary production of streams. The degradation of the adjacent vegetation can also have an impact on the biotic compartment of streams. Particularly, periphyton communities are altered in terms of their composition, abundance, and diversity when the watershed natural conditions are degraded. Diatoms are highly sensitive to changes in the physical and chemical nature of aquatic ecosystems, especially to the concentration of nutrients, changes in acidity, salinity, flow, water transparency or other alterations linked to anthropogenic activities. Recently, water quality monitoring methods in rivers and streams are based in evaluating changes in the diatom composition rather than in physical or chemical parameters. Hence, diatoms can be excellent bioindicators of water quality. Evaluating changes in the diatom community composition can provide key information about environmental changes that occur in a river. Studies bases on diatoms as bioindicators of water condition in mountain tropical ecosystems are rare. In this chapter, I wanted to determine if an alteration of the adjacent vegetation would result in clear-cut changes in the diatom community composition of five mountain streams in southern Ecuador (Loja). The sampled streams show different levels of degradation in their watersheds. I depicted differences in the water condition according to the type of vegetation in the watershed. Moreover, the diatom communities responded to changes in land use in the watersheds. Diatom communities from stream zones with the natural vegetation cover were as different from each other as communities from different streams.
Finally, **Chapter VI** presents the major findings of the thesis. It shows how all chapters fit together and contribute to the field and put the different parts of my thesis into a wider perspective.
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CHAPTER II

Limnological aspects of the Tres Lagunas system (Ecuador)

Alonso Cartuche, Patrick Venail

*Paper to be submitted to Water* [https://www.mdpi.com/journal/water](https://www.mdpi.com/journal/water)

**Abstract**

The Andean wetlands are strategic ecosystems particularly affected by climate change and human interventions. The *páramo* is a wetland ecosystem of high-altitude mountains in the northern Andes (Peru, Ecuador, Colombia, Venezuela) and Central America, located over 3000 meters above sea level. Tropical high-altitude lakes of glacial or volcanic origin are common in the páramos and provide key ecosystem services, such as water storage, regulation of the water cycle and provisioning of freshwater for consumption and irrigation. Despite their importance, tropical high-altitude lakes are still largely unexplored. Despite an increasing interest over the last few years, their environmental characteristics and their biotic composition are poorly documented. Here, we explored and described for the first time a group of 24 high-altitude tropical freshwater lakes located in southern Ecuador (Saraguro/Yacuambi) on a páramo at an average altitude of 3,300 meters above sea level. This study aims to characterize the physical, chemical, trophic and phytoplanktonic characteristics of these lakes. We hope this study will provide a baseline for future studies on the impact of climate change and human activities on tropical high-altitude freshwater lakes.
Introduction

Biomes all around the world are suffering from the impact of human driven activities such as climate change and biodiversity loss, but the full extent of the repercussions is still poorly understood. The Andes are warming faster than many other regions [1,2] and human activities are becoming more intense [3,4]. Despite the importance of tropical high-altitude lakes as natural freshwater reservoirs for human consumption and irrigation purposes, lake studies have been marginalized, with most aquatic studies being focused on rivers and coastal areas. From 1985 to 2015, studies dealing with the physical and/or chemical characterization or their phytoplankton communities in Ecuadorian high-altitude lakes were rare. A recent interest in describing such lakes emerged due to mounting concerns about the consequences of climate change [5-8] and other anthropic activities such as the introduction of fish [9]. A better basic description of these underexplored ecosystems may set a baseline for future studies on the consequences of human driven activities on the structure and functioning of these lakes.

In terms of physical, chemical structure and functioning, tropical high-altitude lakes (hereafter TRHALs) are different from lowland or temperate lakes. This means that TRHALs require specific studies and that findings from temperate or low altitude lakes should not be extrapolated. Some general features of TRHALs shared with high altitude temperate (alpine) lakes are: low average water temperature, extreme diel water temperature variations, strong winds, and intense solar (UV) radiation and low nutrient levels. On the contrary, some specific features of TRHALs include: low seasonality, no ice cover, weak and unstable thermal stratification (cold polymictic), high dissolved organic carbon levels and constant intense solar (UV) radiation through the year. THRALs are almost permanently covered by clouds and drizzles, leading to low average temperatures e.g [10-12]. They were initially described as weakly stratified or polymictic [10,13] but some recent reports suggest that due to temperature increase and reduced winds, these lakes are tending to become more stratified [6,12]. Some studies in THRALS reported that they usually have low to neutral pH, low conductivity, natural low nutrient concentrations, high oxygen levels [5,11,14,15]. Regarding phytoplankton, THRALS form Ecuador are often described as low productive [5,10,12,14]. Moreover, phytoplankton diversity and community composition are in general low but highly variable in space and time [5,8,9,11]
The “Tres Lagunas” wetland is a high-altitude tropical ecosystem located in the eastern range of the southern Ecuadorian Andes in the Black River’s paramo. As in many other Ecuadorian high-altitude ecosystems, several environmental problems are starting to affect the system Tres Lagunas, including road construction, forest fires, agricultural and livestock activities and extreme sports. A third order road (Saraguro-Yacuambi road) running through the system was opened in 2011. During the construction, no actions to mitigate the potential impacts were considered and no environmental impact studies were ever made. The environmental consequences of the road are still unknown [16]. Fires are becoming more frequent, especially in dry seasons. As the páramo vegetation burns, ashes and dust decrease soil water-retention properties. When fires occur, large amounts of carbon dioxide (CO₂) are released, increasing global warming and altering soil properties such as water regulation [17]. To date, scarce scientific studies have been performed in the Tres Lagunas system and some NGOs and a few undergraduate studies have started to explore the area [18]. All agree on the importance of the system for local communities and its contribution to the sustainability of the region and argue that the only way to preserve this ecosystem is through a protected area that includes the lakes. A survey performed in two lakes (Tres Lagunas and Laguna Grande), [19] established a good water quality index (ICA) which was 0.80 in the Tres Lagunas tributary and 0.83 in the effluent. ICA in the Laguna Grande effluent was 0.78. The main goal of this study is to describe the basic limnological aspects of the lakes in the Tres Lagunas system. This information may representant a key base information for future studies. We quantified a series of physical and chemical variables in this group of unexplored high-altitude tropical lakes. We also estimated phytoplankton’s abundance, diversity and community composition.

Methods

Location

The Tres Lagunas wetland or paramo located in Southern Ecuador Andes (Figure 1). harbors approximately 75 lakes of different sizes where several streams are born. Some are in the Amazon mountain range, heading to the Pacific Ocean and other in the Andean mountain range, heading to the Atlantic Ocean [18]. The three larger lakes are Condorshillu, Tres Lagunas and Laguna Grande. The lakes are located at an average
The altitude of 3323 meters above sea level and are encircled by a páramo of ~40 km² [20]. The average air temperatures range between 8 and 10°C, with strong diel temperature variations and a relative humidity higher than 80% [19]. The high humidity value is due to high precipitation levels, water vapor (mist) and low evaporation levels. There is no clear information on actual precipitation values because the wetland is located at the top of the mountain with very irregular topography. However, data from a close weather stations shows that the highest precipitation values occur in March and lower values between July and August [21]. Winds from the Amazon basin generate high levels of precipitation that can exceed 4000 mm/year [22]. Soils are very thick, black and red and erosion levels are low. In addition, soils have very high water retention levels that can exceed 100% and soil pH is frequently very low (~5.0) [22].

![Figure 1](image.png)

**Figure 1**: Location of the Tres Lagunas lake system.

**Studied lakes**

We included 24 high altitude tropical lakes with variables areas. Eleven lakes are in the Amazon (eastern) mountain range and thirteen in the Andes (western) range. The
fieldwork described below was performed in November 2016. The areas of the lakes were estimated using satellite imaging.

**Bathymetric and morphometric analysis of major lakes**

We used a wooden boat to navigate the larger lakes when possible. This included *Laguna Grande, Condorshillu, Tres Lagunas 1, Tres Lagunas 2*. We used a GARMIN CHIRP 72 V sonar coupled with CHIRP (Compressed High-Intensity Radiated Pulse) technology to determine maximum depth and to plot bathymetric maps of the major lakes. Other lakes were too shallow for navigation and bathymetric exploration.

**Sampling**

We determined a sampling route of the system by analyzing maps that included the geographical location, altitude, lake surface, and distances between lakes and from the main road. *In situ*, we used multipurpose probes to collect data on chlorophyll-a concentration (bbe fluoroprobe, mg l⁻¹), dissolved oxygen (mg l⁻¹), redox potential (mv), conductivity (µS/cm), pH and water temperature (°C). Variables were measured at 0.5 m below surface as close as possible to the center of each lake. We measured all variables once. For nutrient analysis, we used acid washed 10 ml plastic tubes to collect water samples at 0.50 m below surface and as far from shore to avoid shallow waters. We preserved the water samples with 98% sulfuric acid. For phytoplankton analysis, we collected samples in 100 ml acid washed plastic bottles at 0.5 m below surface, far from the shore as possible and preserved the samples with glutaraldehyde. All samples were immediately stored in dark and cold conditions, brought to Loja and then to Geneva (Switzerland) by plane. Sampling of the 24 lakes took four complete days (8th to 11th November 2016). In the deepest lake *Laguna Grande*, we also collected temperature and chlorophyll-a concentration data along the water column near the deepest part of the lake.

**Sample analysis**

Nutrients analyses that included total phosphates (µg l⁻¹) and nitrites/nitrates (µg l⁻¹) were performed on an AQ2 discrete analyzer based on EPA 365.1 method, version 2 and EPA 353.2, version 2 (1993) methods respectively. Phytoplankton abundance was determined
based on the [23] protocols. We used an inverted microscope with 40x magnification for cell counting. Phytoplankton samples previously had to be concentrated (volume 30 ml). The samples settled in plastic tubes for 24 hours. For cell counting, we used the Sedgwick-Rafter camera cell counter (S-R Camera). Five transects of 20 fields of view were defined in each one, in total there were 100 fields of vision for each sample/lake. A field of vision measures 0.38 mm². We took pictures of each field of vision, which were used later for phytoplankton identification at the Phylum level. Microalgae biovolume analysis (ml per ml of water) was calculated using geometric forms of microalgae and mathematical equations to get accurate cell volume [24]. Cell density readings (in cells per ml) were determined as well. Species richness was calculated by counting the number of life forms in each sample. In addition, we also estimated the Shannon Biodiversity Indexes based on species number and biovolume. Professor Miriam Steinitz-Kannan (Northern Kentucky University) and professor Kalina Manoylov (Georgia College and State University) supervised and validated the taxonomic identification at the Phylum level. In certain cases, identification to the genus or species level was possible.

Data analysis

In this study, we focus on a purely descriptive approach. We performed a correlation-based Principal Component Analysis (PCA) to reveal potential links among the variables and to determine which variables explained most the differences among lakes. We established the composition of the phytoplankton, based on the prevalence of seven major taxa: Chlorophyta, Cyanobacteria, Bacillariophyta, Pyrrophyta, Chrysophyta, Euglenophyta and Cryptophyta. Unidentified organisms were reported as unknown. We used JMP (SAS, version 14.0.0) for the statistical analyses.

Results

General characteristics of the lakes

During this study, the area of the 24 lakes ranged from 0.51 to 12.41 hectares, with an average surface of 2 hectares (20000 m² or 4.942 acres). Some lakes were close to the road while others were over 3 kilometers far from the road. According to GPS data,
range of altitudes above sea levels of the lakes ranged from 3288 to 3362. Water temperatures were relatively cold, with a minimum of 10.7°C and a maximum of 15.29°C. pH was acidic (average 4.36) but some lakes showed values as low as 3.45. The highest pH value was 5.45. Details on other chemical variables can be found in Table 1.

Table 1: Overview of the geographic, physical, chemical and biological variables

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<th>Variable</th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean</th>
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</tr>
<tr>
<td>Nitrates (mg l⁻¹)</td>
<td>0.0015</td>
<td>0.0194</td>
<td>0.0080</td>
</tr>
<tr>
<td>Chlorophyll-a (µg l⁻¹)</td>
<td>1.49</td>
<td>5.05</td>
<td>3.01</td>
</tr>
<tr>
<td>Total Biovolume (µm³ ml⁻¹)</td>
<td>3.63</td>
<td>241.67</td>
<td>32.39</td>
</tr>
<tr>
<td>Abundance (ind. ml⁻¹)</td>
<td>4066</td>
<td>120914</td>
<td>16563</td>
</tr>
<tr>
<td>Genus richness</td>
<td>15</td>
<td>43</td>
<td>26.75</td>
</tr>
<tr>
<td>ShannonABUNDANCE</td>
<td>0.41</td>
<td>1.18</td>
<td>0.888</td>
</tr>
<tr>
<td>ShannonBIOVOLUME</td>
<td>0.057</td>
<td>0.939</td>
<td>0.521</td>
</tr>
</tbody>
</table>

\[ H' = - \sum_{i=1}^{S} p_i \ln (p_i) \]

We obtained Shannon diversity index values based on both genus and biovolume (formula above). Regarding the biodiversity index based on biovolume, \( S \) is the density of the cell biovolume (ml³ of cells.ml⁻¹), \( p_i \) is the proportion or relative biovolume of
each taxon to total phytoplankton biovolume, and Σ is the sum of density of the cell biovolume.

Regarding phytoplankton related variables, chlorophyll-a values ranged from 1.49 to 5.05 mg per liter. The abundance of phytoplankton showed variation over three orders of magnitude, ranging from over 4000 cells per ml to up to over 120 000 cells per ml. Similarly, phytoplankton biovolume also varied in such proportions, from 3.63 to 241.67 in millions of cubic micrometers of biovolume per milliliter of water. In average, this means that 32 units of volume out of one million represented phytoplankton biovolume.

The number of genera per lake ranged from 15 to 43. Shannon diversity indexes based on abundance and biovolume showed also large variation amongst lakes.

**Morphometry and bathymetry of the four major lakes**

*Laguna Grande* is the largest and deepest of the lakes we sampled in this system of tropical high-altitude lakes from Southern Ecuador. Its estimated water volume is over 250000 cubic meters (*Table 2*). The other three larger lakes were not deeper than 7 meters. Together, *Tres Lagunas 1* and *2* have a total volume close to Condorshillu’s, which is the second deepest and larger of the sampled lakes.

**Table 2**: morphometric variables of the four largest lakes

<table>
<thead>
<tr>
<th>Morphometric variables</th>
<th>Laguna Grande</th>
<th>Condorshillu</th>
<th>Tres Lagunas 1</th>
<th>Tres Lagunas 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estimated area (m²)</td>
<td>119248</td>
<td>63280</td>
<td>38915</td>
<td>44501</td>
</tr>
<tr>
<td>Estimated volume (m³)</td>
<td>250848</td>
<td>173847</td>
<td>83244</td>
<td>96726</td>
</tr>
<tr>
<td>Maximum depth (m)</td>
<td>8.76</td>
<td>6.68</td>
<td>6.16</td>
<td>5</td>
</tr>
</tbody>
</table>

The bathymetric maps of these four lakes revealed very irregular shapes (*Figure 2*). *Laguna Grande* for instance, has a deeper sector located North-east (over 8 meters) and a much shallower area located west (< 4 m, *Figure 2a*). *Condorshillu* has a deeper area near the center (> 6 m) but also has large areas between 3 to 4 meters deep (*Figure 2b*). *Tres Lagunas 1* has a conical shape with a deeper point near its center (*Figure 2c*). Tres Lagunas 2 has a deeper area in the west and the rest of it is shallower (< 3 m, *Figure 2d*).
Figure 2a. Laguna Grande

Figure 2b. Condorsrhillu
Figure 2c. Tres Lagunas 1

Figure 2d. Tres Lagunas 2

Figure 2: Bathymetry of Laguna Grande, Condorshillu, Tres Lagunas 1 and Tres Lagunas 2, respectively. Blue represents deeper areas, brown represents shallower areas. Color scales are not the same across the four figures.
Temperature and chlorophyll-a profile in Laguna Grande

In *Laguna Grande*, close to the deepest point of the lake, the temperature decreased from 13°C at the subsurface to 11°C near the bottom, revealing some weak thermal stratification (Figure 3). The chlorophyll-a profile showed three maxima, at 3 meters, above 5 meters and below 5 meters. Two peaks corresponded to increments in the concentration of chlorophytes and one to an increment in diatoms. Peaks in chlorophyll-a at those depths are often described in high altitude lakes as phytoplankton tends to avoid high UV irradiation closer to the surface. The other taxa showed a small maximum closer to the surface.

![Figure 3](image.png)

*Figure 3:* Temperature (°C, left panel) and Chlorophyll-a (µg/l, right panel) profiles near the deepest point in Laguna Grande. The bbe fluoroprobe instrument used can separate the contribution of three major taxa to total chlorophyll-a concentration. Black = total chlorophyll-a, green = chlorophyte’s contribution, red = diatoms, yellow = other taxa. Error bars represent standard deviations among replicated measurements at each depth.
Links amongst variables

A first principal component analysis (PCA) that included all the geographical, physical, chemical and biological variables reported two first components that explained only 37.9% of the variation in the data (Figure 4). This suggests the absence of strong links amongst variables. Axis one is strongly positively related to chlorophyll-a, whereas axis 2 is strongly positively related to conductivity. As illustrated by the PCA, some variables were positively correlated to each other (Table 3a). Chlorophyll-a concentration was strongly correlated to oxygen, biovolume and abundance of phytoplankton were also strongly correlated. Other biological variables were not completely independent from each other (Table 3a). Finally, pH decreases with the altitude. Some variables were negatively correlated to each other (Table 3b), especially the ones related to phytoplankton. A reduction in chlorophyll-a as the surface of the lakes increased was also observed.

Figure 4: A principal component analysis (PCA) including seventeen variables: four geographic in black (latitude, longitude, altitude and log surface), seven physical and chemical in blue (conductivity, nitrates/nitrites, oxygen concentration, pH, redox potential, temperature (water) and total phosphates), six biological data related to phytoplankton in green (Chlorophyll-a, Log abundance, Log biovolume, genus richness, Shannon index based on abundances, Shannon index based on biovolumes).
Table 3a: Coefficients of correlations amongst variables collected from the 24 lakes. Only correlation coefficients over 0.5 and with p < 0.05 are shown.

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>Coefficient of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll-a</td>
<td>Oxygen</td>
<td>0.7248</td>
</tr>
<tr>
<td>Log BIOVOLUME</td>
<td>Log Abundance</td>
<td>0.6583</td>
</tr>
<tr>
<td>Shannon BIOVOLUME</td>
<td>Shannon ABUNDANCE</td>
<td>0.5926</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>Log BIOVOLUME</td>
<td>0.5139</td>
</tr>
<tr>
<td>Altitude</td>
<td>pH</td>
<td>0.5017</td>
</tr>
</tbody>
</table>

Table 3b: negative coefficients of correlations amongst variables collected from the 24 lakes. Only correlation coefficients over -0.5 and with p < 0.05 are shown.

<table>
<thead>
<tr>
<th>Variable 1</th>
<th>Variable 2</th>
<th>Coefficient of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shannon ABUNDANCE</td>
<td>Log ABUNDANCE</td>
<td>-0.6619</td>
</tr>
<tr>
<td>Log BIOVOLUME</td>
<td>Shannon BIOVOLUME</td>
<td>-0.6582</td>
</tr>
<tr>
<td>Shannon ABUNDANCE</td>
<td>Log BIOVOLUME</td>
<td>-0.5277</td>
</tr>
<tr>
<td>Chlorophyll a</td>
<td>Log SURFACE</td>
<td>-0.5042</td>
</tr>
</tbody>
</table>

Composition of phytoplankton communities

The taxonomic composition analysis (Figure 5) revealed the presence of the major taxonomic groups of phytoplankton: green algae (Chlorophyta), diatoms (Bacillariophyta), cyanobacteria (Cyanobacteria), dinoflagellates (Pyrrophyta), golden algae (Chrysophyta), Euglenophyta and Cryptophyta. Four phyla (greens, cyanobacteria, diatoms and dinoflagellates) were present in all the lakes. Chrysophytes were found in eighteen lakes, Euglenophytes only in seven and Cryptophytes in only one lake. Despite the large variation in the taxonomic composition among lakes, overall, diatoms were the most dominant taxa with over 33% of total biovolume, followed by dinoflagellates (30%), greens (13.5%), cyanobacteria (11.8%), Chrysophytes (over 5%), Euglenophyta (1.5%) and Cryptophytes (0.02%). The overall percentage of unclassified taxa was below 5%.
Figure 5: Phyla composition of total biovolume of phytoplankton at each lake. Lake 1 is Laguna Grande, 19 is Condorshillu, 27 is Tres Lagunas 1 and 28 is Tres Lagunas 2.

Three lakes were largely dominated by a single group (over 70% of the total biovolume). Lake 32 was largely dominated by diatoms (82% of biovolume), lakes 14 and 31 were dominated by dinoflagellates (80% and 73% respectively). There was a dominance of greens in lakes 3 and 24 with 63% and 42% respectively. Cyanobacteria dominated lakes 4, 7 and 9 with 52%, 48% and 36% respectively. The biovolumes of lakes 18, 19, 21, 23, 27 and 30 were dominated by dinoflagellates. Diatoms dominated lakes 1, 6, 10, 12, 17, 28 and 29. The proportions of some major taxa were weakly related to any geographical, physical or chemical variables (coefficients of correlations below 0.5). Only the percentage of diatoms showed a consistent decrease as the redox potential increased.

Discussion

The oldest scientific reports of the general characteristics of tropical high-altitude lakes in Ecuador are almost 40 years old [10,11]. After a couple decades with almost no studies (but see [13]), interest in Ecuadorian tropical high-altitude lakes has rapidly increased over the last decade [5-8,12,14]. This growing interest is especially due to worries about the consequences of climate change or other human activities such as fish introduction. The information and data presented in this study are mostly for descriptive purposes. They
may serve as a baseline for future studies interested in evaluating the impact of human activities on the characteristics of these lakes.

In general, the physical, chemical and biological variables estimated in Tres Lagunas are within the ranges of previous studies under similar geographic conditions in high-altitude mountain lakes from Ecuador. Regarding water temperature, the Tres Lagunas lakes are cold. This corresponds well to previous studies reporting very similar temperatures ranging from 7 to 17°C. Colder lakes were often located at higher altitudes and warmer lakes at lower altitudes [5,6,10-12,14]. The sampled lakes in Tres Lagunas are acidic with some pH values below 4. Whereas in previous reports from TRHALs pH values were closer to neutrality [5,10,11,14], some lakes in the paramos called “turberas” can have pH values below 5, due to the presence of high decomposing organic matter and humic acid [15,25]. In accordance with other similar lakes in the Ecuadorian Andes, the water conductivity in Tres Lagunas was also very low [5,10,11,14], which results from very low ion concentrations [5]. The oxygen levels were high, around 7 mg per liter, as reported [12-14]. The total phosphate levels are in the lower range or below the values reported in other locations [5,12,15] which defines the lakes from Tres Lagunas as oligotrophic or ultra-oligotrophic. This suggests that the Tres Lagunas system has not yet suffered eutrophication due to the influence of human activities. TRHALs have often been described as polymictic, not-stratified or weakly stratified [10-13,15]. Our results from Laguna Grande are in line with such characteristics, showing a slight decrease from 13°C at the surface to 11°C at 7 meters deep. The lakes in the Tres Lagunas system are shallow (<10 m), which certainly contributes to their polymictic characteristics [10,15,25]. However, recent studies have suggested that TRHALs can show thermal stratification that might result from increased temperatures and decreased winds [6,12]. The low levels of chlorophyll-a concentrations reported in Tres Lagunas are also in line with previous reports [5,10,12,14,15]. Paramo lakes are in general less productive than others at lower altitudes, probably due to persistent cloud cover that reduces incident light and limited nutrients [10]. Regarding other phytoplankton related variables, some studies have reported up to 93 and 106 taxa in a single lake [5,13]. The most taxa-rich lake in Tres Lagunas harbored less than half of that number. Our results are in line with other studies reporting from 5 to 80 taxa per lake [9,11,12].
The results of the PCA suggested that chlorophyll-a concentrations and conductivity are the two variables contributing the most to differentiate amongst lakes in *Tres Lagunas*. In a recent similar study across sixteen lakes in the Ecuadorian Andes, Barta and his colleagues found no impact of chlorophyll-a and conductivity as major determinants of variation amongst lakes [5]. Differences are probably due to the incorporation of much colder glacier fed lakes in their study, which resulted to have very different characteristics relative to the non-glacier-fed lakes. Indeed, temperature and distance from glacier were to variables that explained much variance amongst lakes. However, a similar analysis conducted in six lakes from the Cajas National Park showed that conductivity and temperature were important discriminatory variables [14]. Such discrepancies in the results of similar analyses amongst studies underline the specificity of the environmental conditions in each lake that ultimately influence the biotic compartment or vice-versa.

We observed large spatial variation in the taxonomic composition of the phytoplankton communities across the lakes from *Tres Lagunas*. The community composition of phytoplankton assemblages is often used as a natural indicator of water condition and changes in the microbial composition can be signals of disturbance in the physical and chemical environment [26,27]. Recent studies in Ecuadorian TRHALs based on the examination of diatom communities revealed that some have shifted to a new physical and ecological state due to climate change [6,8]. An increase of over one degree Celsius and a significant reduction in wind intensity have generated changes in the stratification regimes of some TRHALs in the Cajas National Park in Southern Ecuador. This altered the ecological conditions of the lakes, allowing previously rare species to become dominant. The *Tres Lagunas* system might soon also be suffering from such changes in the weather conditions and might be exposed to enter new physical or ecological states. More broadly, the Andes are warming fast and pressure from human activities is increasing. We hope the information collected in this study will be used as a baseline for measuring such impacts in the forthcoming years.
References


CHAPTER III

Article

Phytoplankton Diversity Relates Negatively with Productivity in Tropical High-Altitude Lakes from Southern Ecuador
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Abstract

Tropical high-altitude lakes are vital freshwater reservoirs in the Andean regions. They are heavily threatened by human activities that may alter their functioning and hamper the provisioning of key ecosystem services such as water supply. Despite their ecological and social relevance, we know little about these waterbodies, especially regarding the factors influencing their functioning. Here, we explored the links between several environmental variables and phytoplankton productivity, measured as chlorophyll-a concentration and total phytoplankton biovolume. For this, we sampled twenty-four tropical high-altitude lakes located over three-thousand meters above sea level in Southern Ecuador. We found that four abiotic factors combined explained 76% of the variation in chlorophyll-a concentration amongst lakes. Contrary to what studies from temperate regions suggest, taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolume. Moreover, phytoplankton biovolume diversity was negatively correlated to both chlorophyll-a concentrations and total phytoplankton biovolume. This was due to a very uneven distribution of productivity amongst taxa in the more productive lakes. To the best of our knowledge, this is the first attempt to explore the determinants of phytoplankton functioning in tropical high-altitude lakes. We hope that this study will help establishing a baseline for evaluating the consequences of human activities in the ecology and functioning of this vital but fragile ecosystems. Our results suggest that by modifying the abiotic and biotic parameters of tropical high-altitude lakes, human activities can indirectly impact their functioning and their capacity to provide vital ecosystem services.

Keywords: Biodiversity; Ecuador; páramo; phytoplankton; productivity; tropical high-altitude lakes
**Introduction**

High-altitude lakes, also called high-mountain lakes, are important natural freshwater reservoirs for human consumption, irrigation and hydropower production purposes in Andean regions [1–4]. Like high-altitude temperate lakes, tropical high-altitude lakes (hereafter TRHALs) have low average water temperatures. The latter are negatively related to altitude and cloud cover as a determinant of solar incidence. TRHALs also can have extreme diel water temperature variations. They are submitted to strong winds and receive intense solar UV radiations. Generally, but not always, they are low in nutrients [5–8]. Because of their low latitudes, TRHALs have some specific features not shared by high-altitude temperate lakes. This includes moderate or no seasonality, no ice cover, polymictic mixing regimes with often a complex thermal structure, intense UV radiation through the year, high dissolved organic carbon and low UV transparency [2–5,9–13]. All these features make TRHALs unique extreme freshwater ecosystems. One type of TRHALs are the páramo lakes, located above the tree line between approximately from 3200 to 4500 meters above sea level (m.a.s.l.) and filled almost exclusively by rain and groundwater. Another type are the glacial lakes, located at even higher altitudes and are fed directly by glacier melting waters. These two types of lakes can show important differences in both abiotic and biotic characteristics [13]. This study focuses in páramo lakes, located between 3288 and 3362 meters above sea level without a direct connection to glaciers.

TRHALs show a wide range of phytoplankton productivity levels, with chlorophyll-a concentrations ranging from below 1 µg l\(^{-1}\) to values over 8 mg l\(^{-1}\) [4,6,14–17]. Their phytoplanktonic productivity has been negatively related to depth [4], UV radiation [18] and positively to pH [16], total phosphate [4] and total nitrogen [13]. Productivity in TRHALs has often been described as nutrient limited [4,6,14]. Studies of phytoplankton diversity in TRHALs reported over a hundred different genera in different regions of Ecuador [4,13,17]. Regarding the phytoplankton diversity per lake, previous studies counted from 5 to 45 genera per lake in Southern Ecuador [4,13], with taxonomic richness decreasing with altitude and increasing with conductivity [13].

Despite their ecological and social relevance, we still know little about the abiotic or biotic factors influencing the functioning of tropical high-altitude lakes (TRHALs). It is
well established that under controlled experimental conditions, phytoplankton communities with multiple species produce more biomass than monocultures [19–21] and that species richness and biomass are positively related [22]. A recent metanalysis on the importance of species richness for productivity suggests that the above-mentioned effects are even stronger in natural conditions [23]. However, this synthesis only included information from two studies based on phytoplankton communities from temperate regions. To date, observational studies on the relationship between phytoplankton diversity and functioning in freshwater lakes have completely ignored TRHALs (Table 1, [24–51]).

Table 1. Exhaustive list of studies about the relationship between phytoplankton diversity and functioning in freshwater lentic ecosystems around the world. Latitudes in bold represent studies including tropical lakes (i.e., below 23° either South or North). Altitudes in bold represent studies in lakes above 2500 meters above sea level. Notice that no previous study has considered tropical high-altitude lakes. * refers to “lakes” but some studies include other type of waterbodies such as ponds and reservoirs. (n.a) means not available in the original publication.

<table>
<thead>
<tr>
<th>Study</th>
<th>Country</th>
<th>Lake(s)*</th>
<th>Latitude °</th>
<th>Altitude (m.a.s.l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interlandi &amp; Kilham 2001</td>
<td>USA</td>
<td>Jackson, Lewis &amp; Yellowstone</td>
<td>43N–44N</td>
<td>2064-2372</td>
</tr>
<tr>
<td>Grover &amp; Chrzanowski 2004</td>
<td>USA</td>
<td>Joe Pool &amp; Eagle Mountain</td>
<td>32N-33N</td>
<td>162-198</td>
</tr>
<tr>
<td>Ptacnik et al. 2008</td>
<td>Finland, Norway, Sweden</td>
<td>ca. 500 lakes</td>
<td>55N-65N</td>
<td>n.a.</td>
</tr>
<tr>
<td>Das et al. 2008</td>
<td>Canada</td>
<td>Elk, Shawnigan &amp; Sooke</td>
<td>48N</td>
<td>60-183</td>
</tr>
<tr>
<td>Striebel et al. 2009</td>
<td>Germany</td>
<td>46 lakes</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Hogsden et al. 2009</td>
<td>USA</td>
<td>Little Rock</td>
<td>46N</td>
<td>500</td>
</tr>
<tr>
<td>Kruk et al. 2009</td>
<td>Uruguay</td>
<td>18 lakes</td>
<td>33S-35S</td>
<td>0-5</td>
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<tr>
<td>Vogt et al. 2010</td>
<td>Canada</td>
<td>65 lakes</td>
<td>48N</td>
<td>n.a.</td>
</tr>
<tr>
<td>Korneva 2010</td>
<td>Russia</td>
<td>9 reservoirs &amp; 7 lakes</td>
<td>49N-59N</td>
<td>10-120</td>
</tr>
<tr>
<td>Korhonen et al. 2011</td>
<td>Finland</td>
<td>100 lakes</td>
<td>59N-66N</td>
<td>n.a.</td>
</tr>
<tr>
<td>Stomp et al. 2011</td>
<td>USA</td>
<td>540 lakes &amp; reservoirs</td>
<td>27N-49N</td>
<td>1-2753</td>
</tr>
<tr>
<td>Pomati et al. 2012</td>
<td>Switzerland</td>
<td>Zurich</td>
<td>47N</td>
<td>406</td>
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<tr>
<td>Borics et al. 2012</td>
<td>Hungary</td>
<td>26 lakes</td>
<td>n.a.</td>
<td>n.a.</td>
</tr>
<tr>
<td>Fornarelli et al. 2013</td>
<td>Australia</td>
<td>Fitzroy falls reservoir</td>
<td>345</td>
<td>60-822</td>
</tr>
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<td>Weyhenmeyer et al. 2013</td>
<td>Sweden</td>
<td>205 lakes</td>
<td>56N-69N</td>
<td>n.a.</td>
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<tr>
<td>Palffy et al. 2013</td>
<td>Hungary</td>
<td>Balaton</td>
<td>46N</td>
<td>105</td>
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</tbody>
</table>
The uniqueness of tropical high-altitude lakes suggests that previous findings from temperate lakes about the relationship between phytoplankton diversity and functioning might not be extrapolated. The main purpose of the current study is to depict potential links between phytoplankton biodiversity and productivity, quantified as chlorophyll-a concentration and total phytoplankton biovolume. In addition, we aimed to explore if other abiotic variables, independently or in addition to biodiversity, relate to phytoplankton productivity. For this, we examined a set of twenty-four tropical high-altitude shallow lakes located over 3280 meters above sea level in Southern Ecuador.

Materials and Methods

Study system

*Tres Lagunas* belongs to a tropical high-altitude wetland ecosystem called *páramo* [1]. It is located at the eastern range of the southern Ecuadorian Andes, at approximately 20 km from Saraguro and 95 km from Loja, at the border of the Oña and Zamora-Chinchipe provinces (Figure 1).
Figure 1. Location of the Tres Lagunas wetland system in southern Ecuador.

This ecosystem harbors hundreds of small shallow freshwater lakes where rivers in the Amazon mountain range (heading to the Pacific Ocean) and the Andean mountain range (heading to the Atlantic Ocean) begin. The lake system Tres Lagunas consists of around 75 shallow lakes, including three larger ones: Condorshillu (6.3 ha), Tres Lagunas (8.5 ha) and Laguna Grande (12 ha). For the present study, we included the three larger lakes (as major freshwater reservoirs) and another 21 smaller lakes that were randomly selected in the map. The 24 lakes had areas ranging from 0.5 to 12 ha, maximum depths from 1 to 9 meters and altitudes above sea level ranging from 3288 to 3362 meters. Eleven of these lakes are in the Amazon (eastern) mountain range and thirteen in the Andes (western) range. The GPS coordinates of the center of the Tres Lagunas system are 3° 35' 50 " S and 79° 3' 46 " W.

In situ analyses and sampling

The fieldwork described in this study was performed in November 2016. In situ, we collected data on total chlorophyll-a concentration (µg l⁻¹) with a BBE Moldaenke
fluoroprobe. Dissolved oxygen (mg l⁻¹), redox potential (mV), conductivity (µS/cm), pH and water temperature (°C) were measured at the same locations with a HQ40D HACH® portable multiprobe. All the mentioned variables were measured once for each lake near its center. Water samples for nutrient analyses were collected in 10 ml plastic acid-washed tubes at 0.5 m subsurface depth near the center of the lakes on the same days as the in situ measurements. We preserved the water samples with 98% sulfuric acid for further chemical analyses. For phytoplankton analyses, we collected samples in 100 ml acid-washed plastic bottles at 0.5 m subsurface depth near the center of the lakes. We preserved them with 1% final concentration glutaraldehyde, neutralized to pH 7 with NaOH. All samples were immediately stored in the dark, under cold conditions and sent by plane to the laboratory to be analyzed. Due to the harsh access conditions of the wetland and to the remoteness of some lakes, the in situ measurements and water samplings of the 24 lakes took four complete days (from November 8th to 11th 2016). All measurements and water samplings were performed from 10 AM to 3 PM.

Ex situ laboratory analyses

Total phosphate (µg l⁻¹) and dissolved nitrite/nitrate (µg l⁻¹) quantifications were performed in the laboratory on an AQ2 discrete analyzer, based on EPA 365.1 version 2 and EPA 353.2 version 2 methods respectively. Phytoplankton abundances were determined using an inverted microscope with 40x magnification. Phytoplankton from water samples was first concentrated via sedimentation in 50 ml Falcon tubes for 24 hours. For cell counting, we used the Sedgwick-Rafter camera cell counter and included 100 fields of view for each sample/lake. A field of vision measured 0.38 mm². We took digital pictures of each field of vision for further phytoplankton identification and counting. For each taxon, we estimated the average cell biovolume (in µm³) using at least fifty individuals (see Supplementary Material for some examples on the estimations of average cell biovolumes). In each sample, the biovolume of each taxon was calculated as the product of the average cell biovolume by its cell density (in cells per ml). Total phytoplankton biovolume (biovolume of algae per volume of water, µm³ ml⁻¹) was calculated as the sum of the biovolumes of all the taxa present in the sample. Phytoplankton richness in each sample was calculated by counting the number of different taxa at the genus level. As another measure of phytoplankton diversity, we
estimated the Shannon’s diversity index ($H'$) based on the biovolumes of each genus in each lake/sample. The Shannon’s diversity index was calculated with the following formula:

$$H' = - \sum_{i=1}^{S} p_i \ln (p_i)$$

Where $S$ is the density of the cell biovolume (ml$^3$ of cells.ml$^{-1}$), $p_i$ is the proportion or relative biovolume of each taxon to total phytoplankton biovolume, and $\Sigma$ is the sum of density of the cell biovolume. Thus, we refer to this variable as phytoplankton biovolume diversity. The value of the phytoplankton biovolume diversity increases with the number of genera and with the evenness in the contribution of each genus to total phytoplankton biovolume. This is, samples with only a few taxa contributing in large proportion to total biovolume would have a low phytoplankton biovolume diversity (low Shannon’s index based on biovolumes). On the contrary, samples with most taxa contributing in similar proportions to total biovolume would have a high phytoplankton biovolume diversity (high Shannon’s index based on biovolumes). Professor Miriam Steinitz-Kannan (Northern Kentucky University) and doctor Kalina Manoylov (Georgia College and State University) supervised and validated the taxonomic identification of the taxa.

**Data analyses**

Our dataset included four geographic variables: lake surface (in hectares, ha), altitude (in meters above sea level, m.a.s.l.), latitude (in degrees), longitude (in degrees); seven physico-chemical variables: water temperature (in °Celsius), pH, redox potential (in mV), conductivity (in S/m), oxygen concentration (in mg l$^{-1}$), total phosphate (in µg l$^{-1}$), dissolved nitrites/nitrates (in µg l$^{-1}$) and four biological variables: taxonomic richness (number of genera), phytoplankton biovolume diversity (no units), chlorophyll-a (in µg l$^{-1}$) and total phytoplankton biovolume (in µm$^3$ ml$^{-1}$, which represents the biovolume of phytoplankton in µm$^3$ per ml of lake water). We used chlorophyll-a concentrations and total phytoplankton biovolume as two proxies of phytoplankton productivity. We ran linear-models linking single or multiple of the above-mentioned variables to phytoplankton productivity (i.e., chlorophyll-a and total biovolume separately). We then used the AIC (Akaike information criterion) to determine the abiotic (chemical, physical,
geographic) or biotic variables that better described chlorophyll-a and total phytoplankton biovolume. We used JMP (SAS, version 14.0.0) for all statistical analyses. Total phytoplankton biovolume and surface of lakes were log transformed to improve the normality of the data.

**Results**

Chlorophyll-a concentrations in the lakes from *Tres Lagunas* ranged from 1.49 to 5.05 µg l⁻¹, with an average concentration of 3.01 µg l⁻¹. Total phytoplankton biovolume spanned over four orders of magnitude, from 34.08 × 10³ to 31.02 × 10⁷ µm³ ml⁻¹, with an average value of 20.1 × 10⁷ µm³ ml⁻¹. Chlorophyll-a concentration and total phytoplankton biovolume (log transformed) were positively correlated across the lakes (*correlation coefficient = 0.514, P = 0.01, N = 24*). Genera richness ranged from 15 to 43 per lake with an average richness of 26.75 genera per lake. The less diverse lake in terms of phytoplankton biovolume diversity had a Shannon’s index of 0.057 whereas the more diverse one had a Shannon’s index of 0.939. The average phytoplankton biovolume diversity (Shannon’s) index of the lakes was 0.521. These two measures of phytoplankton diversity (genera richness and phytoplankton biovolume diversity) were positively related (*correlation coefficient = 0.343, P = 0.1, N = 24*), but would still encompass different aspects of phytoplankton’s diversity.

Four abiotic variables correlated well to chlorophyll-a concentration (*Table 2*). This included total phosphate concentration (*Figure 2*), oxygen concentration and altitude, that related all positively to chlorophyll-a concentrations. This means that lakes with higher phosphate concentrations, more dissolved oxygen levels and located at higher altitudes showed higher chlorophyll-a concentrations. The percentages of variance in chlorophyll-a concentrations explained by total phosphate, oxygen and altitude were 53%, 31% and 19% respectively. The surface of the lakes (log transformed) correlated negatively to chlorophyll-a, meaning that smaller lakes had higher chlorophyll-a concentrations than larger lakes. Only total phosphate concentrations related well and positively to total phytoplankton biovolumes (i.e., log biovolume, *Figure 2, Table 2*).
Table 2. List of abiotic and biotic variables (related variables) that correlated significantly with either chlorophyll-a or total biovolume (response variable). Variables are ranked from more positive to more negative correlation coefficients and includes only variables with P-values below 0.1.

<table>
<thead>
<tr>
<th>Response variable</th>
<th>Related variable</th>
<th>Correlation coefficient</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll-a</td>
<td>Total Phosphate</td>
<td>0.725</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Chlorophyll-a</td>
<td>Oxygen</td>
<td>0.559</td>
<td>0.004</td>
</tr>
<tr>
<td>Chlorophyll-a</td>
<td>Altitude</td>
<td>0.436</td>
<td>0.033</td>
</tr>
<tr>
<td>Log biovolume</td>
<td>Total Phosphate</td>
<td>0.429</td>
<td>0.037</td>
</tr>
<tr>
<td>Chlorophyll-a</td>
<td>Shannon (Biovolume)</td>
<td>-0.393</td>
<td>0.058</td>
</tr>
<tr>
<td>Chlorophyll-a</td>
<td>Log Surface</td>
<td>-0.504</td>
<td>0.012</td>
</tr>
<tr>
<td>Log biovolume</td>
<td>Shannon (Biovolume)</td>
<td>-0.658</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Figure 2. Correlations between total phosphates (phosphates, in µg l⁻¹) and chlorophyll-a concentrations (µg l⁻¹, left panel) and between total phosphates and total phytoplankton biovolume (log biovolume in 10³ µm³ ml⁻¹, right panel). Indicated statistics are the correlation coefficients (ρ) and P-values. Grey lines represent linear fits.

In other words, lakes with higher phosphate concentrations had also higher total phytoplankton biovolumes. Together, the correlation analyses suggest that phytoplankton production in the Tres Lagunas system, quantified as chlorophyll-a and total biovolume, might be partially phosphorous limited.

The two measures of phytoplankton biodiversity, this is genera richness (taxa richness) and Shannon’s diversity index (phytoplankton biovolume diversity), showed different relationships with chlorophyll-a concentrations and total phytoplankton biovolumes (Table 2, Figure 3). Taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolumes. On the contrary, phytoplankton biovolume diversity (measured as Shannon’s diversity index based on biovolumes) was negatively correlated
to both chlorophyll-a concentrations and total phytoplankton biovolumes (Figure 3). This suggests that lakes dominated by fewer genera had higher chlorophyll-a concentrations and total biovolume levels than lakes with more even distributions of biovolume amongst different taxa. Regarding the negative relationship between phytoplankton biovolume diversity and total biovolume (Figure 3, low right hand panel), a closer look at data allowed us depicting some interesting trends. We noticed the presence of three groups of lakes based on their total phytoplankton biovolumes. The first group includes three lakes with values of total phytoplankton biovolume above $1.10^6 \mu m^3 ml^{-1}$ and Shannon’s diversity index values spanning from very low to intermediate. The analysis of the taxonomic composition of each lake showed that the communities of the two lakes with highest total biovolumes were largely dominated by *Mougeotia*, a filamentous alga (family: Zygnemataceae). This genus represented up to 96% of the total biovolume and 61% of the cell counts. The lake with the third highest total biovolume was largely dominated by *Peridinium*, a dinoflagellate representing 72% of the total biovolume and 74% of cell counts. This information is clearly showing that an extremely uneven distribution of biovolume amongst the different taxa explains the combined high total biovolume and low biovolume diversity values of these three lakes. The second group of lakes includes nine waterbodies with total phytoplankton biovolumes ranging from $0.5.10^4$ to $1.10^6 \mu m^3 ml^{-1}$ and with Shannon indexes varying from low to intermediate/high. Overall, these lakes also reported a large dominance of biovolume production (up to 95%) by the dinoflagellate *Peridinium*, or by colonial diatoms such as *Synedra*, *Fragilaria* or *Asterionella*. However, these taxa did not over-dominate cell counts, with percentages of total abundance ranging from 25 to 40%. Finally, a group of twelve lakes had biovolume values below $0.5.10^4 \mu m^3 ml^{-1}$ and phytoplankton biovolume diversities spanning from intermediate to high. In these lakes, the above-mentioned genera represented less than 70% of the total biovolume and less than 38% of the cell counts. In lakes from groups 2 and 3, the genus *Mougeotia* was not recorded at all.
Figure 3. Correlations between genus richness (left panels), phytoplankton biovolume diversity (Shannon’s diversity based on biovolume, right panels), chlorophyll-a (in µg l⁻¹, upper panels) and total phytoplankton biovolume (log biovolume in 10⁴ µm³ ml⁻¹, lower panels). Indicated statistics are the correlation coefficients (ρ) and P-values of the correlation. Grey lines represent linear fits for P values below 0.1.

Overall, the analysis of the composition of phytoplankton communities suggests that the observed negative relationship between total biovolume and phytoplankton biovolume diversity (Figure 3) can be largely explained by a very uneven distribution of biovolume amongst taxa. Total phytoplankton biovolume in the samples from Tres Lagunas decreased as the dominance, both in biovolume and cell counts, of some taxa such as Mougeotia and Peridinium decreased. Because the contribution of each taxa to total chlorophyll-a could not be determined, we can only speculate about the reasons why chlorophyll-a decreases as phytoplankton biovolume diversity increases. Based on the distribution of the data from the positive correlation between total phytoplankton biovolume and chlorophyll-a concentration, it is quite possible that the uneven contribution of taxa to total biovolume is at the origin of a negative correlation between
biovolume diversity and chlorophyll-a concentration. Five lakes were characterized with low biovolume diversity but very high chlorophyll-a values, three of which also showed the highest biovolume levels that resulted from the dominance of one single taxon.

After fitting linear models with all possible combinations of single and multiple factors (both abiotic and biotic) to chlorophyll-a concentrations and total phytoplankton biovolumes, we ranked these models according to the Akaike criteria (AICc, Table 3).

Table 3: Summary table of the different linear models (single and multiple factor) linking abiotic and biotic variables to chlorophyll-a. Models are ranked by increasing AICc (Akaike information criterion) values. Oxygen stands for oxygen concentration, altitude is for altitude above sea level.

<table>
<thead>
<tr>
<th>Factors included in model</th>
<th>R²</th>
<th>P-value</th>
<th>AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phosphate, oxygen, altitude, log surface</td>
<td>0.777</td>
<td>&lt; 0.0001</td>
<td>50,764</td>
</tr>
<tr>
<td>Total phosphate, oxygen, log Surface</td>
<td>0.724</td>
<td>&lt; 0.0001</td>
<td>52,249</td>
</tr>
<tr>
<td>Total phosphate, altitude</td>
<td>0.684</td>
<td>&lt; 0.0001</td>
<td>52,322</td>
</tr>
<tr>
<td>Total phosphate, oxygen, altitude, log Surface</td>
<td>0.719</td>
<td>&lt; 0.0001</td>
<td>52,707</td>
</tr>
<tr>
<td>Total phosphate, altitude, log Surface</td>
<td>0.717</td>
<td>&lt; 0.0001</td>
<td>52,877</td>
</tr>
<tr>
<td>Total phosphate, oxygen</td>
<td>0.628</td>
<td>&lt; 0.0001</td>
<td>56,198</td>
</tr>
<tr>
<td>Total phosphate, log Surface</td>
<td>0.586</td>
<td>&lt; 0.0001</td>
<td>58,776</td>
</tr>
<tr>
<td>Oxygen, altitude</td>
<td>0.525</td>
<td>&lt; 0.0001</td>
<td>59,166</td>
</tr>
<tr>
<td>Oxygen, log Surface</td>
<td>0.578</td>
<td>0.0001</td>
<td>59,244</td>
</tr>
<tr>
<td>Oxygen, altitude, log Surface</td>
<td>0.602</td>
<td>0.0003</td>
<td>61,099</td>
</tr>
<tr>
<td>Oxygen</td>
<td>0.312</td>
<td>0.0045</td>
<td>68,062</td>
</tr>
<tr>
<td>Altitude, log Surface</td>
<td>0.379</td>
<td>0.0067</td>
<td>68,52</td>
</tr>
<tr>
<td>Oxygen, altitude</td>
<td>0.375</td>
<td>0.0071</td>
<td>68,659</td>
</tr>
<tr>
<td>Log Surface</td>
<td>0.254</td>
<td>0.012</td>
<td>70,011</td>
</tr>
<tr>
<td>Altitude</td>
<td>0.190</td>
<td>0.0331</td>
<td>71,984</td>
</tr>
</tbody>
</table>

For chlorophyll-a concentration, the linear model that better described variation in the data (77.7%, with the lowest AICc value) included four abiotic factors: total phosphate, oxygen, altitude and log surface. As shown previously with the correlations, the best single abiotic predictor of chlorophyll-a concentration was total phosphate, explaining 52.5% of the variation amongst lakes. None of the biotic variables included in our study appeared in the models that better predicted chlorophyll-a concentrations. For total phytoplankton biovolume, the model that better described variation in the data (50.3%, with lowest AICc, Table 4) included total phosphate concentration and phytoplankton biovolume diversity (Shannon’s index based on biovolumes). The best single predictor of total phytoplankton biovolume was phytoplankton biovolume diversity (Shannon’s diversity), explaining 43.3% of the variation in total biovolume among lakes.
Table 4. Summary table of the different linear models (single and multiple factor) linking abiotic and biotic variables to total phytoplankton biovolume. Models are ranked by increasing AICc (Akaike information criterion) values. Only models with p-values below 0.05 are presented.

<table>
<thead>
<tr>
<th>Factors included in the model</th>
<th>R²</th>
<th>P-value</th>
<th>AICc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total phosphate, Shannon’s diversity</td>
<td>0.503</td>
<td>&lt; 0.001</td>
<td>63,223</td>
</tr>
<tr>
<td>Shannon’s diversity</td>
<td>0.433</td>
<td>&lt; 0.001</td>
<td>63,466</td>
</tr>
<tr>
<td>Total phosphate</td>
<td>0.184</td>
<td>0.0367</td>
<td>72,226</td>
</tr>
</tbody>
</table>

Discussion

The levels of chlorophyll-a measured in this group of lakes from *Tres Lagunas* are comparable to most previous studies in tropical high-altitude lakes (TRHALs) from Mexico, Bolivia and other parts of Ecuador [6,13,15–17]. Like in a recent study in the Cajas National Park in Southern Ecuador [4], chlorophyll-a values correlated positively with total phosphate concentrations. However, another study with similar levels of phytoplankton production involving TRHALs in Ecuador found that total nitrogen concentration was the only variable that explained some variation in chlorophyll-a amongst lakes [13]. Other abiotic variables such as pH [16] and UV radiation [18] have been related to chlorophyll-a concentrations in TRHALs as well. Such discrepancies amongst studies in terms of the abiotic variables that relate with phytoplankton’s productivity (with chlorophyll-a as a proxy) suggest that the large geographic variation in the productivity of TRHALs can hardly be predicted by one single abiotic factor. In line with this hypothesis, our analysis showed that chlorophyll-a concentrations had simultaneous positive and negative links with several abiotic variables, including phosphate concentration, oxygen concentration, altitude and lake surface. According to the results of the linear models, these four abiotic variables together explained 78% of the variation in chlorophyll-a levels amongst the lakes from *Tres Lagunas*. In brief, our results showed smaller lakes located at higher altitudes, with higher concentrations of oxygen and total phosphates tend to be more productive in terms of chlorophyll-a concentrations.

Despite the positive correlation of chlorophyll-a with total phytoplankton biovolume observed in our dataset, the links between the different abiotic variables and total biovolume were weak. Among all the abiotic variables included in this study, only total phosphate concentration related to total phytoplankton biovolume, explaining just 18%
of its variation (versus 52% for chlorophyll-a). This result suggests that other variables not included in our study (abiotic or biotic) might be more relevant as determinants of total phytoplankton biovolume. Previous studies about the determinants of phytoplankton production in tropical high-altitude lakes using total biovolume as proxy are very rare. This can be explained because acquiring total biovolume information requires more sophisticated equipment (e.g., particle counter, cytometer) or time demanding techniques (e.g., microscopy). In a recent study, total phytoplankton biomass was measured along sixteen lakes in the Ecuadorian Andes, but none of the abiotic variables included related to phytoplankton biomass [13]. In our study, only phytoplankton biovolume diversity index strongly improved the capacity of the linear models to predict total biovolume variation among lakes. Alone it was the best single predictor of total biovolume (43.3% of the variation) and together with total phosphate explained up to 50.3% of the variation.

So far, studies on the relationship between phytoplankton diversity and ecosystem functioning in freshwater lakes have overlooked tropical high-altitude lakes. To the best of our knowledge, this study represents the first attempt to link phytoplankton diversity to productivity in these extreme aquatic ecosystems. Our results revealed no relation between taxonomic richness and either chlorophyll-a or total phytoplankton biovolume. A similar null pattern between taxonomic richness and functioning was described before in temperate lakes from Finland [27] but contradicts most studies from temperate lakes that show a positive impact of taxonomic richness on phytoplankton productivity [27,29,35]. Moreover, phytoplankton biovolume diversity, measured as the Shannon’s diversity index based on biovolumes, correlated negatively with both chlorophyll-a and total phytoplankton biovolume. As explained earlier, the Shannon’s diversity index used here was based on the contribution of each genera’s biovolume to total biovolume. In other words, it is a measure of the evenness in the distribution of total biovolume across the different genera in a sample or lake. Total biovolume can relate to biovolume diversity via two non-exclusive mechanisms. First, via a more even distribution of biovolumes across taxa (higher Shannon’s index). This would result in a positive correlation between phytoplankton biovolume diversity and total biovolume. Second, by having only a few taxa contributing largely to total biovolume, which results in lower Shannon’s index values. This would result in a negative relationship between total biovolume and biovolume diversity, as observed in our dataset.
Whereas not included as one of the factors explaining much variation in chlorophyll-a amongst lakes, phytoplankton biovolume diversity turned out to be the best single predictor of total phytoplankton biovolume. The analysis of community composition revealed that this pattern was mainly due to changes in the dominance of a few taxa amongst lakes, both in terms of abundance and biovolume. A reduction in the prevalence of taxa such as Mougeotia (a filamentous algae) and Peridinium (a dinoflagellate) resulted in a concomitant increase of biovolume diversity and a decrease of total phytoplankton biovolume. Such negative links between diversity and productivity, due to the dominance of some taxa, are not very frequent but have been reported in temperate lakes [34,41,43,50]. Previous observational studies about the importance of biodiversity for ecosystem functioning in freshwater ecosystems have led to the belief that phytoplankton diversity has a general positive impact on productivity [23]. This means that in order to be more productive, freshwater bodies must contain more species of phytoplankton. However, this conclusion was based on a large dataset that included over 1150 sites from Scandinavia [27] and North-America [46] but containing no single data from high-altitude tropical lakes. Our results offer original empirical evidence suggesting that such effects are not universal. In our set of lakes, the most productive lakes were not necessarily the more diverse ones, suggesting that some conclusions driven from temperate lakes should not be extrapolated to other ecosystems such as páramo lakes. To confirm our findings, we advocate for additional studies testing the influence of phytoplankton diversity on functioning from a wider variety of freshwater ecosystems.

Phytoplankton productivity in the Tres Lagunas lakes showed large geographic variation, due in big part to variation in abiotic and biotic factors, as reported in previous studies from temperate regions [27,31,35,36,46]. Overall, chlorophyll-a was strongly related to four abiotic factors whereas total biovolume was linked to phytoplankton biovolume diversity. However, the scope of these findings has at least two limitations. First, lakes were sampled only once, thus ignoring the spatial and temporal variability in the abiotic and biotic parameters considered. It is possible that the relationships among variables described in this study might vary when considering other spatiotemporal scales [24,35]. Second, the patterns described here are purely correlational because no causal relationships amongst variables can be established using only observational data [27,31]. Our study addresses diversity as a possible determinant of phytoplankton productivity,
but it is well known that the alternate perspective, with productivity as a determinant of diversity can be considered too [24,31,36].

Tropical high-altitude lakes (TRHALs) are the major freshwater reservoirs in Andean regions. They are very vulnerable to human activities, putting at risk their own functioning and the provisioning of key ecosystem services such as water supply [1–4,52–53]. As in other South American high-altitude ecosystems, road construction, controlled fires, agriculture, livestock and extreme sports are modifying the Tres Lagunas ecosystem. To our best knowledge, no environmental impact studies have ever been made in this region and no actions to mitigate their potential impacts have been considered. We hope this study will help establishing a baseline for evaluating some of the future consequences of human activities in the ecology and functioning of this vital but fragile ecosystem. Our results suggest that by impacting abiotic and biotic parameters of these lakes, human driven activities can also have either positive or negative impacts on the functioning of tropical high-altitude lakes and the provisioning of ecosystem services.

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Author Contributions: Conceptualization, P.V.; Methodology, A.C, Z.G. and P.V.; Formal Analysis, A.C. and P.V.; Writing – Original Draft Preparation, A.C.; Writing – Review & Editing, A.C., Z.G, B.I and P.V.; Funding Acquisition, P.V.

Data availability: Data included in this study will be available in the Dryad Digital Repository upon acceptance.

Conflicts of Interest: The authors declare no conflict of interest.
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CHAPTER IV

Environmental, not geographic distance relates to phytoplankton community structure across high-altitude tropical lakes.

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Paper in preparation

Abstract

Tropical high-altitude lakes are vital freshwater ecosystems for the functioning and dynamics of the paramos. They play a major role in the hydrogeological cycle and provide important hydrological services such as water storage. Describing the patterns and processes of community composition in these lakes is required to better understand the consequences of their degradation by human activities. In this study we tested the geographical and environmental components of the distance decay relationships in the phytoplankton structure across 24 tropical high-altitude lakes from Southern Ecuador. Phytoplankton composition at the phyla level showed high among-lake variation in the tropical high-altitude lakes from Tres Lagunas. We found no links between the geographic distance and phytoplankton composition. On the contrary, we observed some environmental related patterns of community structure. The absence of support for the distance decay relationship observed here can result from a conjunction of local niche-based effects and dispersal limitations. Phytoplankton community composition in the Tres Lagunas system or any other ecosystem may be jointly regulated by niche-based and neutral forces that still need to be explored. Despite not proving a mechanistic explanation for the observed patterns of community structure, we hope our findings will help improving our comprehension on these vulnerable and vital ecosystems. More studies in tropical high-altitude lakes are urgently required.
Introduction

Defining the processes that determine the patterns of community structure is a major challenge in ecology [28]. For decades, community ecologists have generated numerous hypothesis regarding the distribution and coexistence of species within and amongst habitats [29,30]. In this context, the distance decay relationship suggests that similarity amongst communities decreases with geographical distance [31,32]. Geographically distant communities are expected to be more dissimilar in their structure and composition than closer ones. That pattern can be linked to at least two non-exclusive mechanisms. First, communities become less similar with distance because the environmental conditions become less similar. This assumes a strong correlation between geographic distance and environmental dissimilarity. In community ecology this niche-based process is also called “species sorting” [33]. Second, the dispersal capacity of living organism is limited. This means that even if the environment does not change over space, the limited capacity of an organism to disperse does not allow it to succeed everywhere. Thus, an organism has major chances to be found closer to its original localization rather than far from it [34]. In opposition to species-sorting, this second mechanism considers that organisms perform equally in any environmental conditions (e.g., neutrality). Both forces can contribute to generate a distance decay pattern in community composition [35]. This means that a distance decay pattern alone cannot be used as evidence of the underlying mechanisms. Indeed, actual changes in community structure over space and distance result from a balance between local niche-based processes and regional dispersal capabilities [36].

For microorganism, a niche-based perspective called the Baas-Becking’s hypothesis dominated the literature for decades [37,38]. It suggests that given their small size microorganisms are ubiquitous, but the local environment determines where they can persist. This means that only the capacity of a microorganism to cope with the different local environmental conditions, not its dispersal capabilities, would determine its spatial distribution patterns. Consequently, distance decay relationships in microorganisms may result from a strong correlation between geographic and environmental distance. Species sorting would be the dominant underlying mechanism. However, evidence collected over the last two decades suggests that microorganisms show biogeographical patterns, with both local (e.g., species sorting) and regional (e.g., dispersal) processes being important
As for macroorganisms, the distance decay relationships in microorganisms is jointly influenced by local environmental filters and dispersal [32,45,46].

In microalgae, the evidence for local and regional processes dominating community structure is mixed [41,45-50]. A study with diatoms from finish streams found limited support for species sorting. Also, spatial effects overcame environmental variables as drivers of community structure [41]. On the contrary, algal communities in lakes and reservoirs from Greece showed a strong correlation with environmental conditions [46]. Something similar was observed amongst diatom communities in another study in Finland, where community structure was more controlled by niche-related than spatial factors [45]. Two other studies showed that algal community structure was controlled simultaneously by both spatial and environmental factors [47,48]. Regarding the relationship between geographical distances and community similarity in microalgae, some studies found evidence of distance decay relationships [45,48,50] but some others did not [46]. Such discrepancies can be explained because factors such as the spatial scale or the environmental context influence the distance decay relationship in microalgae [47,49]. Moreover, studies also offer mixed evidence on the impact of environmental distance on community structure similarity in freshwater phytoplankton. Some studies support the relationship [45,47,49] but some others do not [48].

Community ecology studies with microalgae from tropical lakes are extremely rare. We know little about the patterns of distribution of microalgae in such freshwater ecosystems. Studies on patterns and processes of community composition in high altitude ecosystems are required to better understand the consequences of their degradation by human activities, including climate change. In this study we tested the geographical and environmental components of the distance decay relationships in the phytoplankton structure across 24 tropical high-altitude lakes from Southern Ecuador. We hope our findings will help improving our comprehension on these vulnerable and vital ecosystems.
Methods

Studied lakes

The *Tres Lagunas* system is a group of tropical high-altitude lakes located in Southern Ecuador at an average altitude of 3323 meters above sea level. For this study, we included 24 lakes with wide ranges of physical, chemical and biotic characteristics separated from a hundred meters to up to six kilometers from each other (Cartuche et al., in preparation).

Sampling and physicochemical analyses

We used multipurpose probes to collect data on chlorophyll-a concentration (bbe fluoroprobe, mg l⁻¹), dissolved oxygen (mg l⁻¹), redox potential (mv), conductivity (µS/cm), pH and water temperature (°C). Variables were measured at 0.5 m below surface as close as possible to the center of each lake. We measured all variables once. For nutrient analysis we used acid washed 10 ml plastic tubes to collected water samples at 0.50 m below surface and as far from shore to avoid shallow waters. We preserved the water samples with 98% sulfuric acid. All samples were immediately stored in the dark and cold conditions, brought to Loja and then to Geneva (Switzerland) by plane. Sampling was performed from 8th to 11th November 2016. Total phosphates (µg l⁻¹) and nitrites/nitrates (µg l⁻¹) were performed on an AQ2 discrete analyzer based on EPA 365.1 method, version 2 and EPA 353.2, version 2 (1993) methods respectively.

Phytoplankton composition

For phytoplankton analysis, we collected samples in 100 ml acid washed plastic bottles at 0.5 m below surface, far from the shore as possible and preserved the samples with glutaraldehyde. Sampling was performed from 8th to 11th November 2016. All samples were immediately stored in the dark and cold conditions, brought to Loja and then to Geneva (Switzerland) by plane. We used an inverted microscope with 40x magnification for cell counting. Phytoplankton samples were previously concentrated by letting them to settle in plastic tubes for 24 hours. For cell counting, we used the Sedgwick-Rafter camera cell counter (S-R Camera). Five transects of 20 fields of view were defined in each one,
in total there were 100 fields of vision for each sample/lake. A field of vision measures 0.38 mm². We took pictures of each field of vision, which were used later for phytoplankton identification. Microalgae biovolume analysis (ml per ml of water) was calculated using geometric forms of microalgae and mathematical equations to get accurate cell volume [24]. Cell density readings (in cells per ml) were determined as well. We established the composition of the phytoplankton community of each lake based on the prevalence of six major taxa: Chlorophyta, Cyanophyta, Bacillariophyta, Pyrrophyta, Euglenophyta and Cryptophyta. Unidentified organisms were reported as unknown and represented less than 2% of the total abundance in every lake. The unknown algae were not included in further analyses.

**Community dissimilarity**

We used two methods for measuring the phytoplankton community dissimilarity among lakes in terms of their phyla composition based on their relative contribution to total biovolume. First, we calculated the inversed Bray-Curtis community similarity index between each pair of lakes. Using the inversed Bray-Curtis index allowed interpreting higher values of the index as an indicator of higher dissimilarity amongst lakes in their phytoplankton community structure. Second, we performed a cluster analysis based on the phyla composition of lakes and then calculated the Euclidean distances separating each pair. A higher Euclidean community distance represents a higher community dissimilarity. We used JMP (SAS) software version 14 to perform the clustering and to estimate the community structure distances. The two measures of community similarity were highly correlated ($\rho = 0.886, p < 0.0001, n = 276$). For simplicity, only results based on Euclidean community distances are presented. Results with Bray-Curtis similarity provided almost identical results.

**Geographical distances**

We calculated the geographical distances in meters amongst lakes using maps and data on latitude, longitude and altitude from the center of each lake.
Environmental distances

We included seven variables to estimate environmental distances amongst lakes: conductivity, nitrates/nitrites, oxygen concentration, pH, redox potential, temperature (water) and total phosphates. For this, we first performed a cluster analysis based on the physical and chemical variables and then calculated the Euclidean distances separating each pair. A higher Euclidean distance represents a higher environmental dissimilarity. We used JMP (SAS) software version 14 to perform the clustering and to estimate the Euclidean-based environmental distances.

Data analysis

We first performed a correlation-based Principal Component Analysis (PCA) to reveal potential links among the variables and to determine which variables explained most the differences among lakes. The PCA included seventeen variables: two morpho geographic (surface area and altitude), seven physical and chemical (conductivity, nitrates/nitrites, oxygen concentration, pH, redox potential, temperature (water) and total phosphates) and eight biological variables related to phytoplankton (Chlorophyll-a, total biovolume, %Chlorophyta, %Bacillariophyta, %Cyanophyta, %Chrysophyta, %Euglenophyta and %Pyrrophyta). To test the relationships between environmental, geographic and community structure distances (dissimilarity), we fitted linear and binomial models to the data using JMP version 14 (SAS).

Results

Links amongst variables

The principal component analysis (PCA) reported a first component positively related to chlorophyll-a that explained 23.9% of the variation in the data and a second component positively related to altitude and pH that explained 14.8% of the variation in the data (Figure 1). As shown by the PCA, some community structure variables (i.e., percentages of each phyla) were correlated to environmental variables (Table 1). The contribution of diatoms (%Bacillariophyta) to total biovolume decreased with the redox potential and
with altitude above sea level. The contribution of cyanobacteria (% Cyanophyta) to total biovolume decreased with temperature. Finally, the contribution of Euglenophytes to total biovolume increased with total phosphates. A strong negative correlation between the percentage of diatoms and the percentage of dinoflagellates (%Pyrrophyta) was observed ($\rho = -0.608$, $p < 0.001$), which means that an increase in the biovolume of diatoms was at the detriment of the biovolume of dinoflagellates. No other strong correlations were observed amongst the relative contribution of each phyla to total biovolume.

![Figure 1: Principal component analysis (PCA) including seventeen variables: two geographic (altitude and surface), seven physical and chemical (conductivity, nitrates/nitrites, oxygen concentration, pH, redox potential, temperature (water) and total phosphates) and eight biological variables related to phytoplankton (Chlorophyll-a, total biovolume, % Chlorophyta, % Bacillariophyta, % Cyanophyta, % Chrysophyta, % Euglenophyta and % Pyrrophyta). Dots represent lakes.](image-url)
Table 1: coefficients of correlations amongst composition variables (contribution of each phyla to total biovolume) and environmental variables. Only correlation coefficients over 0.45 and with p < 0.05 are shown.

<table>
<thead>
<tr>
<th>Structure variable</th>
<th>Environmental variable</th>
<th>Coefficient of correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Bacillariophyta</td>
<td>Redox potential</td>
<td>-0.592</td>
</tr>
<tr>
<td>% Bacillariophyta</td>
<td>Altitude</td>
<td>-0.466</td>
</tr>
<tr>
<td>% Cyanophyta</td>
<td>Temperature</td>
<td>-0.466</td>
</tr>
<tr>
<td>% Euglenophyta</td>
<td>Total phosphate</td>
<td>0.452</td>
</tr>
</tbody>
</table>

Phyla community structure

We found high variability in the phyla composition of the high-altitude tropical lakes from Southern Ecuador (Figure 2). The number of phyla (from 4 to 6) and their relative contributions to total biovolume in each lake determined their structure similarities. Some lakes were dominated by only one phylum, while others were more equally structured. Lake 31 was isolated in the clustering due to the higher contribution of Euglenophyta to total biovolume and almost a total absence of Cyanophyta and Chlorophyta.

Figure 2: Cluster analysis of the phyla community structure of the 24 lakes. Numbers in the middle represent lakes. The percentages represent the contribution of each phyla to total biovolume.
We found no evidence of a relationship between geographic distance and the composition dissimilarity (Figure 3a, $R^2 = 0.0048$, $p = 0.246$, $n = 276$) but found weak but positive linear and binomial relationships between environmental distance and composition similarity (Figure 3b, linear: $R^2 = 0.0162$, $p = 0.034$; binomial: $R^2 = 0.033$, $p = 0.001$, $n = 276$). The binomial model explained twice the variance in community similarity compared to the linear model. The geographic and environmental distances from our dataset were not linearly related ($R^2 = 0.0002$, $p = 0.81$) but a binomial model provided a good fit ($R^2 = 0.034$, $p = 0.008$). A model with environmental and geographic distance together did not provide a better fit to the data than environmental distances alone ($R^2 = 0.021$, $p = 0.0523$).

Discussion

The aim of the current study was to characterize the spatial structure of phytoplankton community structure in tropical high-altitude lakes and to explore its relationships with geographical and environmental distances. We found large among-lake variation in the structure of phytoplanktonic assemblages at the highest taxonomic level in the Tres Lagunas system. The number of phyla and their relative contributions to total biovolume were spatially highly variable. Our results show that the phytoplankton composition of the lakes was related to the environmental distances. Community dissimilarity at the phyla level was maximal at intermediate environmental distances. In other words, the
composition dissimilarity of phytoplankton increases from low to intermediate environmental distances but drops when the environments are more dissimilar. Previous studies with freshwater phytoplankton also found relationships between environmental distance and community dissimilarity [45-47,49]. However, these studies focused on community variation at much lower taxonomic levels (e.g., species) and reported either linear or logarithmic patterns. Differences in the taxonomic resolution used may impede our results to be comparable to previous studies.

Our analyses of the influence of individual environmental variables on community composition (i.e., PCA) revealed that redox potential, altitude, water temperature and total phosphorous were the variables that contributed most to the variability in phytoplankton structure. An increase in the redox potential and altitude above sea level were detrimental for the contribution of diatoms (the two environmental variables were positively correlated). The increase in temperature was unfavorable for cyanobacteria contribution whereas increasing total phosphate benefited the contribution of euglenophytes. Previous studies also reported effects of environmental variables such as pH, conductivity, total phosphates, temperature and phytoplankton community composition [45,48]. Any generalization across different ecosystems and environmental conditions is unwarranted, especially when comparing studies performed in very different conditions. The environmental conditions of tropical high-altitude lakes are unique and differ much from the ones reported elsewhere in temperate and low land freshwater ecosystems. It can be expected that the environmental variables that matter for phytoplankton in tropical high-altitude conditions differ from those at other altitudes and latitudes.

We found no evidence of any relationship between geographic distance and community similarity. It is possible that the spatial structure of communities in Tres Lagunas is different at large spatial scales, where dispersal limitation may act more strongly. Perhaps at relatively small spatial scales like in this study (<10km) high dispersal between local communities homogenizes the spatial structure of the communities and a higher impact of environmental conditions is observed. Microorganisms’ biogeographical patterns are probably stronger at much larger spatial scales [51]. In line with our results, other previous studies with freshwater phytoplankton found no support for the distance decay relationship in freshwater phytoplankton [46,47,49]. Importantly, this lack of support of the
distance decay relationship cannot be interpreted as unequivocal evidence for considering microorganisms are ubiquitous. In this study, we estimated geographical distances as overland GPS-based distances between lakes with different levels of water connectivity, belonging to two different drainage basins. It is possible that a higher spatial signal in community structure would be found if we had included water course distances or other determinants of dispersal capabilities in phytoplankton. However, connectivity patterns in *Tres Lagunas* would be hard to establish because paramos are wetlands where the soil is permanently soaked with water. The lack of a distance related spatial structure in our study may also result from the sampling methods used. We sampled each lake only once at one depth. Thus, our dataset included only a snapshot of the transient community structure of phytoplankton in these lakes, without including within-lake or temporal variability. Single sampling may only represent a very small proportion of the real diversity of a lake [52].

Despite the observed relationships between environmental variables and community composition, a major proportion of among-lake community variation remained unexplained. Some possible explanations include that other important environmental variables were not included, that the spatial scale we used does not embrace the regional processes that matter for phytoplankton, that trophic effects such as grazing by zooplankton were not considered and that the taxonomic resolution (i.e., phyla level) was too coarse. Phytoplankton includes a huge variety of life forms and it is possible that the environmental and spatial forces driving distribution patterns differ across the major phyla. Moreover, recent studies in similar tropical high-altitude lakes show that phytoplankton communities in these lakes are quite sensitive to human driven alterations such as eutrophication, temperature increase and the introduction of fish [5,8,9]. Tropical high-altitude lakes can also show significant temporal variations in community composition [12]. All the above-mentioned factors might contribute and interact with each other to determine the phytoplankton community composition in tropical high-altitude lakes.

Phytoplankton composition at the phyla level showed high among-lake variation in the tropical high-altitude lakes from *Tres Lagunas*. We found no links between the geographic distance and phytoplankton composition. On the contrary, we observed some environmental related patterns of community structure. Spatial composition patterns
based on field data alone like the ones reported here cannot be used to reveal the underlying mechanistic determinants of community assembly. Controlled experiments are required to depict the processes that generate those patterns. The absence of support for the distance decay relationship observed here can result from a conjunction of local niche-based effects and dispersal limitations. Community composition in the Tres Lagunas system or any other ecosystem may be jointly regulated by niche-based and neutral forces that still need to be explored. More studies are required to determine their relative importance as drivers of community composition in unexplored systems such as the high-altitude tropical lakes.
References

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CHAPTER V

Changes in periphyton communities with land use in tropical mountain streams from Loja (Ecuador).

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Paper in preparation

Abstract

The vegetation along a stream regulates the flow of sediments and nutrients. It also helps stabilizing the riverbanks and controls the watershed microclimate that ultimately influences the primary production of rivers. The degradation of the adjacent vegetation of streams can also have an impact on the biotic compartment of rivers. Particularly, periphyton communities are altered in terms of their composition, abundance and diversity when the natural conditions in the watershed are degraded. Diatoms are highly sensitive to changes in the physical and chemical nature of aquatic ecosystems, especially to the concentration of nutrients, pH, salinity, flow, water transparency or other alterations linked to anthropogenic activities. Hence, diatoms can be excellent bioindicators of water quality. Nowadays, some water quality monitoring methods in streams are incorporating changes in the diatom composition in addition to traditional physical or chemical parameters. Evaluating changes in the diatom community composition can provide key information about environmental changes that occur in a river. While widespread in Europe and North America, studies based on diatoms as bioindicators of water condition in mountain tropical ecosystems are rare. In this study, we wanted to determine if an alteration of the adjacent vegetation would result in significant changes in the diatom community composition of seven streams in southern Ecuador. The sampled streams show different levels of degradation in their watersheds. Changes in the diatom composition could be used as signals of degradation in water condition due to land use alterations. We depicted some differences in the physico-chemical water condition parameters according to the type of vegetation in the watershed. Moreover, the diatom communities also responded to changes in land use in the watersheds. Diatom communities from zones with different land use within a stream were as different from each other as communities from different streams.
**Introduction**

Lotic ecosystems are key components of the hydrological cycle. Rivers and streams in good conditions in urban areas offer multiple ecological benefits linked to social welfare and economic development. They also protect populations against natural hazards, such as floods, runoff control and the effects of climate change [53]. Unfortunately, nowadays rivers are severely affected by pollution related issues. Among the main pollution sources are direct discharge of domestic wastewater, industrial waste dumping and the excessive use of agrochemicals in agriculture. These pollution factors are affecting the biotic component of lotic freshwater ecosystems, their hydrological, physical and chemical characteristics, hampering their capacity to provide hydrological functions and services. It is well established that the surrounding vegetation (e.g., riparian vegetation) regulates important processes in aquatic ecosystems, such as inputs of light, organic matter and nutrients [54]. Water quality is influenced by the decomposition of riparian plants which release nutrients and organic matter to water, and by the exudation of various organic and mineral components [55]. The surrounding herbaceous vegetation in rivers can also help stabilizing the riverbanks and regulating the flow of sediments and nutrients [56] Trees and shrubs can generate a microclimate that influences the primary production of rivers [57]. Riverine forests maintain important physical and biological resources and fulfill ecological functions through the interaction of soils, hydrology and biotic communities [58]. The alteration or degradation of the surrounding areas (i.e., land use) also affects living organisms that inhabit rivers [59-61]. Particularly, periphyton communities are altered in terms of composition, abundance, and diversity [62]. Loss of adjacent vegetation increases the light availability for periphyton communities, leading to an increased biomass production [63,64].

Diatoms are the dominant group of algae in aquatic ecosystems [65] and are responsible for the most part of the primary production in shallow rivers [66]. Diatoms are highly sensitive to changes in the chemical nature of aquatic ecosystems, especially to the concentration of nutrient such as phosphorous, nitrogen, silica and to the presence of contaminants [67]. When the water condition is altered by changes in acidity, salinity, nutrient concentration, flow, water transparency or other alterations linked to anthropogenic activities, changes in diatom community composition are key to evaluate changes in the environment [68]. In tropical rivers, the structure and dynamic processes
of diatoms communities are controlled by flow variations too [69]. Diatom are used for biomonitoring the ecological quality or ecological status of lotic ecosystems, replacing in some cases the traditional methods [70,71]. Moreover, ranges of environmental conditions and tolerances are known for most species [72]. Therefore, the evaluation of changes in the diatom community composition can provide precise information about environmental changes that occur in a river [73].

The objective of this study was to determine whether an alteration of the adjacent vegetation in streams results in changes in the diatom community composition of mountain streams in southern Ecuador (Loja). Three of these streams, located within a protected area, are part of the water network that supplies drinking water to approximately 270 000 people. These streams show different levels of degradation in their watersheds linked to differences in land use that we reported as forested, open and degraded. The forested areas are dominated by the natural vegetation and show no clear visual signs of altered land use. The open areas are zones where the natural vegetative cover has been replaced by pastures or crops. The degraded areas show clear signs of human intervention by the presence of livestock, urbanized areas of garbage dumping. Given the well documented sensitivity of diatoms to changes in the environment, we expect important changes in their community composition as a direct consequence of changes in land use. We also expect diatom water condition in open zones to show some signs of degradation, with a tendency to move towards the water condition of the degraded areas. Ultimately, changes in the diatom community composition could be used as early signals of the degradation of water quality.

Methods

Study area

The study was conducted in seven small Andean streams in the Loja agglomeration. This region is located in a temperate equatorial climate with temperatures between 12 to 16°C [74]. The seven mountain streams are located above 2020 meters above sea level and make part of two small hydrographic systems in the Loja canton: Zamora and Northern Malacatos. They belong to Zamora’s river upper sub-basin, which originates in Cajanuma’s mountains. This hydrographic system is circumscribed within the Santiago
river demarcation that carries its waters to the Amazon watershed. El Carmen, Mónica and Volcán provide water for human consumption. El Carmen supplies about 60% of the population of Loja, around 269017 inhabitants [75]. During the rainy season, it provides around 300 liters of water per second, but in dry seasons this value drops to 180-200 l.s\(^{-1}\) [76].

**Maps**

To elaborate thematic maps, we collected secondary information of free access available in (Military Geographic Institute, IGM 2017) from where we obtained information such as hydrography and digital models amongst other sources (Table 1). The information obtained during the sampling in the field was contrasted with digital cartography at different scales (1:100000; 1:50000; 1:25000, 1:5000) and aerial photography, resources that allowed to generate precise information of the study areas. The technique for the elaboration of base cartography as well as vegetation coverage maps considered the reference system used by the Military Geographic Institute (IGM 2017), defined by the following parameters: Horizontal Datum: WGS84; Reference Zone: Zone 17 South; Cartographic Projection: Universal Transverse Mercator. Soil cover categories and vegetation structure were determined by GIS techniques. The ArcGis 10.5 program was used for data processing and mapping.

**Table 1:** sources and information collected for producing maps of the seven watersheds.

<table>
<thead>
<tr>
<th>CARTOGRAPHIC RESOURCE</th>
<th>SOURCE</th>
<th>SCALE</th>
<th>YEAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrography</td>
<td>SigTierras</td>
<td>1:25000</td>
<td>2015</td>
</tr>
<tr>
<td>MDT (Digital Model of the Territory)</td>
<td>SigTierras</td>
<td>1:25000</td>
<td>2015</td>
</tr>
<tr>
<td>Aerial images</td>
<td>SigTierras</td>
<td>1:5000</td>
<td>2012</td>
</tr>
<tr>
<td>Land Use Coverage</td>
<td>SigTierras</td>
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<td>2018</td>
</tr>
<tr>
<td>Sampling Points</td>
<td>Research team</td>
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<td>2017</td>
</tr>
<tr>
<td>National System of Protected Areas</td>
<td>Ministry of the Environment (MAE)</td>
<td>1: 100000</td>
<td>2017</td>
</tr>
<tr>
<td>Political Administrative Division</td>
<td>Ecuador's National Committee for Internal Borders (CONALI)</td>
<td>1:50000</td>
<td>2017</td>
</tr>
</tbody>
</table>
Sampling design

The fieldwork was carried out with the participation of the Loja National University (Faculty of Agriculture and Renewable Natural Resources). In November 2016 we collected water samples in seven streams located around Loja: *El Carmen, San Simón, Mónica, Volcán, Violeta, Tenería* and *Shucos* (Figure 1). For *El Carmen, San Simón, Mónica* and *Volcán* streams, we identified and delimited two zones. One zone with the natural vegetation cover for at least one hundred continuous meters in the watershed. This zone is called *forested*. The second zone presented clear signs of vegetation clearing and land use in the surroundings (agriculture, livestock, housing, garbage). This zone is called *open* (Figure 2). The samples from the two zones were sampled as close as possible from each other to reduce spatial distances from sampling points. For the other three rivers (*Shucos, Tenería* and *Violeta*) only samples from *open* areas were collected because these streams did not have long continuous sections of natural vegetation cover. Land use maps for each stream can be found as Supplementary Material. In each sample location, a transect of 100 meters along the watercourse was defined. Each transect was divided in five sectors of 20 m each and we collected data and water samples in each of them. Thus, each zone of a stream was sampled five times (i.e., sub-samples).
Figure 1: map of the seven streams included in this study near the city of Loja (Ecuador). Pink dots represent sampling location in each stream. Blue drop symbols represent location of water treatment plants.
Water sampling

Water samples for periphyton community analysis were collected in each sub-sampling point in acid washed 100 ml plastic bottles and conserved with glutaraldehyde (1ml, in a final concentration of 2.5%). Sampling methods based on [77] [78] and [79] were used. Five small rocks were collected from the bottom of the river. We used a natural substrate sampling device with an insulated circular area of 2.9 cm² and a toothbrush to scrape the rocks to get substrate, which was washed with 20 ml of water and collected into the acid washed plastic bottles. At each sub-sample sample point, we used a portable multiparameter probe (HQ40D Hach) to collect information on water temperature, pH, dissolved oxygen and conductivity. Additionally, 10 ml of water samples were collected and stored with sulfuric acid (0.5 ml, at a final concentration of less than 5%) for further total nutrient analysis. Nutrients analyses that included total phosphates (µg l⁻¹) and nitrites/nitrates (µg l⁻¹) were performed on an AQ2 discrete analyzer based on EPA 365.1 method, version 2 and EPA 353.2, version 2 (1993) methods respectively. To measure water flow, we applied the salt tracer method technique suitable for measuring flows in small streams with varying velocities and turbulence [80]. It is a technique independent of river cross-section and distance. Soluble salt increases electrical conductivity (EC) and serves as a tracer for water movement. The method consisted of pouring into a point of
the stream a known amount of salt dissolved in water, and at 40 meters (dissolution distance) down the watercourse we measured the change in conductivity in the water. There is a direct linear dependence between the increase in water conductivity and the amount of soluble salt (EC <1000 µS.cm\(^{-1}\)), which allowed the electrical conductivity (EC) to be converted in an amount of salt that passes through each unit of time. By graphing the time and conductivity data, we obtained an area under the curve that was used to calculate the water flow (\(Q \text{ in } l \text{ s}^{-1}\)) as follows:

\[
Q = \frac{V}{k \ast \sum (EC(t) - EC_{bg}) \Delta t}
\]

with: \(EC(t) = \text{measured conductivity at time } t \text{ in } \mu S \text{.cm}^{-1}\), \(EC_{bg} = \text{measured background conductivity in } \mu S \text{ cm}^{-1}\), \(V = \text{volume of salt injected in mg}\) and \(k = \text{conversion factor in } \frac{mg \mu S}{cm \text{l}}\).

**Periphyton community analysis**

In the laboratory, we quantified periphyton abundance, total biovolume and two aspects of diversity (species richness and Shannon diversity based on species abundance). For the quantitative analysis of periphyton, we used the cell counting Sedgwick-Rafter method which satisfies the quantitative evaluation criteria for a precise estimation and reproducible of periphyton communities [81]. This method employs a slide of rectangular shape (50 x 20 mm x 1 mm depth) representing an area of 1000 mm\(^2\) and volume 1.0 ml. The protocol as proposed by [82] was modified according to our aims and consisted of taking a subsample of 40 ml from the original water sample (100 ml) and to concentrate it sedimentation for 24 hours. Then, a volume of 37 ml of water was extracted from the tube. This volume had to be homogenized and only 1 ml of aliquot was taken for the analysis into the Sedgewick Rafter Counting Chamber. For the estimation of abundance, total biovolume and for identification a 60x lens microscope was used. For the counting, three transects in the slide were defined. All the cells were differentiating between single cell, colonies and filaments. For cyanobacteria counting we followed a protocol proposed by [83], nevertheless, variations were made according to periphyton cell concentration in the samples. For counting non-constrictive filamentous forms of cyanobacteria, at least four cells per filament were measured and we repeated this procedure 20 times.
Furthermore, the average length of these 20 filaments should was estimated. Then, we calculated the mean number of cells per filament, dividing the cellular average length by the average length of the filaments. When it was not possible to measure the cellular length, the average length of the cells available in the scientific literature could be used (the average length of filaments is performed in the same way as above). In this study no cyanobacteria constrict forms or colonies were found. For quantification of green algae species, we used the same procedure is followed for non-constrict cyanobacteria filaments. Diatom identification required first removing organic matter from frustules through an oxidation process using a strong acid and making permanent slides in a high refractive index resin [62]. For this, we followed [84] protocol, modified by [85]. Material preparation for diatom identification was performed at the laboratory of the Department of Biological and Environmental Sciences of Georgia College and State University (USA). For each sampling zone (8 zones in total), 50 ml of sample was first homogenized in 250 ml beakers and then 20 ml were transferred to a beaker for the acid digestion process. For this, we added 20-25 ml of nitric acid to each sample and a few drops of potassium dichromate (99, 5%) as catalyst to accelerate the digestion process. We heated the mixture to 250°C until it started to boil. We gently washed the samples with de-ionized water until pH was neutral. After several washes and obtaining a neutral pH, 50 ml of digested diatoms were transferred to small vials to be homogenized. Then, 25 ml were transferred to glass bottles were the microalgae precipitated for 3 hours. After discarding excessive water, around 3 ml of concentrated washed sample were used for diatom identification. The material was dispersed in duplicate on a 22x22 mm microscopic coverslip. The slide cover assembly was done in an extraction hood by placing a small amount of Naphrax™ (Brunel Microscopes Inc., United Kingdom) on the slide, heating the plate slowly and letting it cool down for 15 seconds. The slide dried overnight before we removed any additional Naphrax. A single random transect was used for the enumeration of diatoms mounted on the microscope slides [84]. The abundance was measured as the number of frustules per ml. When possible, a minimum of 300 valves (150 frustules) was counted, otherwise, a minimum of 100 valves was quantified. Taxa identification to the species level and cell counting was performed with a Leica DM2500 microscope with a Leica DFC295 camera at Georgia College and State University using a 1000x magnification (Leica Microsystems, Wetzlar, Germany). The taxonomic keys used for the identification of diatoms included [86-89], [90-92], [93,94] and [95-98]. For each of the transects in the slide, all the cells found were photographed at a magnification of
To estimate the total number of cells (cells.ml\(^{-1}\)), we estimated the volume of each transect at 8 mm\(^3\). Each one of the samples (independently if they belonged to open or forested) we used 5 sub-samples and three transects in the slide for each sub-sample. Consequently, the total volume analyzed for each sub-sample was 120 mm\(^3\). The taxonomic identification to the species or genus level was performed using standard taxonomic identification techniques [99].

**Data analysis**

Ten variables including chemical, physical and biological information were averaged within each stream and across streams for the forested, open and degraded zones. Data from Carmen, Mónica, San Simón and Volcán are used to compare the open and forested parts of the streams. Data from Tenería, Violetas and Shucos represent a highly degraded watershed. The variables included in this comparison are: total phosphates concentration, nitrates/nitrites concentration, water temperature, conductivity, pH, dissolved oxygen, water flow, total periphyton abundance, periphyton species richness and Shannon index based on species abundances. For each stream we determined the community composition at the phyla level as their relative contribution to total abundance. We considered the four more dominant phyla: Chlorophyta, Cyanophyta, Bacillariophyta and Rhodophyta. We averaged the contribution of each phyla to total abundance across streams and for each type of zone (forested, open and degraded). We performed a correlation-based Principal Component Analysis (PCA) to reveal potential links between the environmental variables and the diatom species composition. For the latter, we included data on the abundance of each species in a stream and zone (forested, open or degraded). The PCA included seven environmental variables: conductivity, oxygen concentration, pH, flow, water temperature, total phosphates and nitrates/nitrites. Thus, the position of a stream and zone in the PCA graph summarizes its environmental characteristics and species composition. We also performed a cluster analysis to estimate and to compare the similarity in diatom community composition from the different streams and zones. Shucos and San Simón were not included in the PCA and cluster analyses because the samples were degraded during transportation before the examination of the diatom communities. We used JMP version 14 (SAS) for data analysis.
Results

Environmental conditions in the different zones of streams

A summary of the physical, chemical and biological variables of the three zones is presented in Table 2. Several variables show a tendency to increase in the open zones compared to the forested zones. This included total phosphates concentration, nitrates/nitrites concentration, water temperature, conductivity, pH, algae abundance and species richness. However, given the large variability among streams, only temperature was significantly higher (t-test, p < 0.05). Other variables showed the opposite trend. Oxygen concentration and water flow were lower in the open zones, compared to the forested zones. Again, the differences were not significant due to variability amongst streams (t-test, p > 0.2). The degraded zones that served as reference showed considerably higher levels of total phosphates, pH and species richness but also reduced conductivity and water flow compared to the open and forested zones. Across the full dataset, some important correlations among environmental variables were observed (i.e., coefficients of correlation > 0.6). For instance, total phosphate was negatively correlated with flow, whereas nitrate concentration was positively linked with conductivity. Temperature was negatively related to the oxygen concentration and pH to water flow as well.

Table 2: Overview of the hydrological, physical, chemical and biological variables of the three different zones. S.D = standard deviation.
**Phyla composition in the different zones**

Diatoms were by far the dominant phyla across all the streams, representing overall 82% of cell counts (Figure 3). Cyanophyta were second (12.4%), followed by Chlorophyta (4.1%) and Rhodophyta (1.8%). We observed high among-stream and among-zone variation in the phyla composition. Some zones only included a single phylum (i.e., Bacillariophyta). None of the sampled zones included all four phyla together. The average composition of the three zones showed also high variability, especially the open and degraded zones were very different from each other. The former included a much higher proportion of cyanobacteria, reported in two streams, *Mónica* and *San Simón*. The degraded and forested zones had overall very similar phyla compositions, except that the forested zones included some rhodophytes and chlorophytes. Only diatoms and cyanobacteria were found in the degraded zones.

![Figure 3: Phyla composition of the periphyton communities in different zones (forested, open and degraded) and different streams, represented as their relative contribution to total abundance. (C = Carmen, M = Mónica, Ss = San Simón, Vo = Volcán, T = Tenería, Vi = Violetas, Sh = Shucos). AVG represents the average composition in each zone. Numbers in the yellow bars represent the proportion of diatoms at each site.](image-url)
Diatom composition in the different zones

We identified a total of 22 genera and 48 species of diatoms (Table 3). The more speciose genera are *Achnanthidium* (5 species), *Gomphonema* (6 species), *Navicula* (9 species) and *Nitzschia* (6 species). Over the full dataset, the most abundant genera are *Gomphonema* (39%) and *Achnanthidium* (22.7%). The more abundant species are *Gomphonema minutum* (23.9%) and *Achnanthidium minutissimum* (14.1%). The ubiquitous genera were *Achnanthidium* and *Gomphonema*, present in seven out of eight sampling sites. The more cosmopolitan species were *Gomphonema sp.* and *Achnanthidium minutissimum* present in six sites. *Achnanthidium subatomoides* was found in 5 sites. The number of genera per site ranged from 3 to 10, and the number of species from 4 to 16. A total of 27 species were only reported in one site each. The number of endemic taxa ranged from 4 to 10 per stream and from 1 to 6 per zone. In the three streams with forested and open zones, the number of taxa exclusive to one zone decreased when the natural vegetative cover along the stream changed from forest to pastures or crops. The variation in the number of species per site and the big differences in their relative contributions to total abundance resulted in Shannon-indexes ranging from very low (0.32) to high (0.96). The cell abundance ranged over two orders of magnitude from c.a. 26000 cells per ml to c.a. 265000 cells per ml, with a gradient of increasing abundance from forested to open and degraded zones.

**Table 3.** List of diatom species identified in each stream and each zone with their respective contributions (as percentage) to total abundance. Empty boxes represent that the species was not recorded.

<table>
<thead>
<tr>
<th>Species</th>
<th>Carmen forested</th>
<th>Carmen open</th>
<th>Mónica forested</th>
<th>Mónica open</th>
<th>Volcán forested</th>
<th>Volcán open</th>
<th>Tenería degraded</th>
<th>Violeta degraded</th>
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</tbody>
</table>
Synedra sp | Ulnaria ulna | 2.0 | 10.1 | 0.7 |
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<td>70628</td>
<td>25874</td>
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<td>0.85</td>
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The two principal components of the PCA (Figure 4) explained 23.9% and 17.2% of the variation in the data respectively (together 41.1%). The first axis is positively related to total phosphates and pH, and negatively related to flow. The second axis is positively related to nitrates/nitrites concentration and conductivity. The sampled sites differed much in their environmental characteristics and community composition. The PCA discriminated well the two degraded sites from the open and forested sites, even though they were very different from each other. Violeta (degraded) was linked to a higher phosphate concentrations and higher water temperatures. Four species seem to benefit from these conditions and are only present of become more abundant in this site (Geissleria decussis, Navicula germainii, Nitzschia recta and Planothidium lanceolatum). Tenería (degraded) is linked to a lower conductivity and nitrates/nitrites concentrations. Other four species benefited from these conditions and are only present or more abundant in this site (Cocconeis placentula, Navicula lanceolata, Nitzschia frustulum and Rhoicosphenia abbreviata). The forested zone in Carmen differentiated well from the other sites by the presence of three species (Achnanthidium rivulare, Kabayasiella subtilissima and Planothidium sp.). Volcán open differentiated from other sites by the dominance of Gomphonema sp. Mónica forested included higher abundance of Achnanthidium minutissimum and unique presence of Bacillaria paradoxa and Nupela sp. The other three sites: Carmen open, Volcán forested and Mónica open also reported unique taxa but had less extreme environmental characteristics.
Figure 4: Principal Component Analysis among sampled sites that included seven environmental variables (blue arrows) and abundance information from 48 diatom species. Streams Carmen (grey), Mónica (green) and Volcán (orange) include open and forested zones. Tenería (brown) and Violeta (red) represent degraded reference streams. Stars represent the position of each stream and zone in the PCA hyperspace. For clarity, only the more conspicuous species are represented.

The cluster analysis based on community composition data confirmed that the communities from the two degraded streams differed most from the other six sites, irrespective of the type of zone (Figure 5a). The open and forested sites were mixed in the cluster-tree configuration. The long terminal branches in the tree (colored branches) confirm that the diatom communities from the eight different sites have considerable composition uniqueness. Moreover, the short basal branches in the tree (grey) suggest that the pool of species and relative abundances that are shared by the different sites is rather low. In other words, most of the variation in spatial community composition among sites comes from taxa being unique to each site (i.e., endemism). This confirms the results from the PCA (Figure 4).
The analysis of community dissimilarity based on the Euclidean distances from the clustering provides two key results (Figure 5b). First, diatom communities from different zones within streams (intra streams) are equally different in their composition than 1) communities from open sites in different streams (inter streams open zones), 2) communities from forested sites in different streams (inter streams forested zones) and 3) communities from the two zones across different streams (open to forested zones). Briefly, intra-stream dissimilarity and inter-stream dissimilarity levels are equal. The second key result is that diatom communities from open and forested zones are both similarly different from the communities in the degraded sites.

![Figure 5: a) Cluster analysis of the community composition of the eight sampled sites. The two degraded sites (in red) from a separate cluster. The other six diatom communities are mixed without a clear clustering pattern. The terminal branches of the tree (in color) are relatively long, suggesting that each site has considerable uniqueness in its composition. The short basal branches (grey) suggest that the pool of species shared by the different sites is reduced. Blue represent open sites and green represents forested sites. b) Comparison of the Euclidean distances separating the different sites according to their diatom species composition. Crosses represent averages, horizontal lines represent median values and bars represent standard deviations. Boxes represent 95% confidence intervals. Intra streams represents diatom communities from different zones within each stream (n = 3). Inter streams open zones represents communities from open sites in different streams (n = 3). Inter streams forested zones represents communities from forested sites in different streams (n = 3). Open to forested zones represents communities from the two zones across different streams (n = 6). Second, that diatom communities from open (open to degraded zones, n = 6) and forested zones (open to degraded zones, n = 6) are similarly different from the communities in the degraded sites.](image)

**Discussion**

The composition of periphyton assemblages, specially diatoms, is very sensitive to changes in the chemical and physical parameters of water but also to changes in land use in proximity to the watercourse. The use of diatoms offers the possibility to detect changes
in water condition that could not be detected using traditional physico-chemical analyses. Whereas diatoms are largely being used as bioindicators of the ecological status of waterbodies in North America and Europe [100,101], periphytic communities from tropical mountain streams are less studied and we know little about the factors influencing their spatial distribution. Moreover, Andean mountain ecosystems are rapidly degrading due to human activities such as agriculture, livestock, mining, dam construction and climate change. We studied a set of seven tropical mountain streams in Southern Ecuador to test if land use differences result in changes in the physico-chemical characteristics of streams and consequently on the periphyton community composition.

The analysis of the physico-chemical characteristics of streams in zones exposed to different land use near Loja reported some patterns. Compared to the areas with native vegetation along the stream (forested), zones without pastures or crops (open) showed a tendency to be moving towards the characteristics of more heavily degraded zones (degraded). This included an increment in total phosphates and temperature that probably lead to a higher algal abundance. The absence of natural forest has been linked to higher nutrient levels, increased temperatures and algal biomass in streams elsewhere [61,63,102-105]. Several other physico-chemical variables did not show that same tendency and the open zones could not be distinguished from forested zones. Our results might be suggesting that the clearing of the natural vegetation in streams near Loja are not leading to major changes in the physico-chemical characteristics of streams yet. However, slight increments in nutrient concentrations, temperature and algal biomass might be indicators of the initial stages of a degradation of water condition.

A major goal of this study was to increase the knowledge about the composition of periphyton assemblages from tropical mountain streams. We found that diatoms dominate the periphyton in the streams around Loja but there is a large spatial variation in community composition within and amongst streams. In terms of phyla composition, the communities from the degraded zones were more similar to the ones from forested zones than to the communities from the open zones. The latter showed a higher prevalence of cyanobacteria and a reduced contribution of diatoms to total abundance. A previous similar study relating land use to diatom community composition in mountain streams in Mexico also found that cyanobacteria were present near pastures but not in forested zones [61]. The increased contribution of cyanobacteria in the open zones of two streams might
be linked to the increased levels of phosphate and higher temperatures. The observed increase in the prevalence of cyanobacteria in the open zones of two streams seems to represent a clear indicator of the degradation of water condition as a consequence of clearing the natural forest in the watershed of tropical high-altitude streams. The degraded zones were less diverse in terms of phyla, with no records of chlorophytes or rhodophytes.

Overall, *Gomphonema* and *Achnanthidium* were the more speciose and dominant genera in the Loja’s streams. These two genera have been reported for being widespread in rivers and streams over a wide range of environmental conditions [106-108]. Other 20 genera with 37 species of diatoms contributed to a high spatial variability among streams and zones within lakes. A total of 27 species were described in only one of the eight sites analyzed. However, none represented more than 14 percent of the total abundance in that site. The majority were rather rare, representing form 0.5 to 4 percent of total diatom abundance only. The number of species per sample site is relatively low, ranging from 4 to 16. This low level of species richness is in accordance with other studies of diatom communities in mountain streams [61]. Whereas the average number of species in the open and forested zones were not different, the number of taxa only present in one site was lower in the open zones than in the forested zones. This suggests that changes in the environmental conditions over a few hundreds of meters were enough to select for species capable of coping with different environmental conditions. Unfortunately, our data does not allow us to determine which variables were responsible for this trend.

The results of the principal component analysis also allowed better understanding some of the large spatial variation in community composition amongst streams. The presence and abundance of some taxa were strongly related to some of the physico-chemical variables included in this study and consequently to some of the sites. For instance, *Volcán open* had four endemic species whose presence was related to high conductivity and nitrates/nitrites. *Violeta degraded* also had four endemic taxa that are related to higher temperatures and total phosphates. The presence or relative abundance of the other unique species from other sites were not related to any physico-chemical variable. Perhaps some other environmental variables not included in our study might relate to their prevalence. Also, trophic effects such as grazing were not considered in our study and may play a major role in defining the distribution and abundance of some taxa.
The principal component analysis also helped to better understand the spatial variation in community composition. It shows that the two degraded sites and the presence of some unique taxa were linked to environmental variables such as pH, temperature, total phosphates and water flow. These physico-chemical variables strongly discriminated between the two degraded and the three other streams along the first axis of the PCA analysis. On the contrary, the spatial variation in community composition between the open and forested zones was better explained by changes in conductivity and nitrate/nitrite concentrations along the second axis of the PCA. However, no consistent pattern of transition from forested to open zones was depicted over the three streams. In Volcán and Carmen the transition from forested to open in terms of community composition and environmental characteristics occurred upwards along the vertical axis of the PCA, whereas the opposite happened in Mónica. This might suggest that the three rivers are under different levels of perturbation or probably in some parameters not considered in this study.

The cluster analysis confirmed the composition uniqueness of all the sites, with few species being cosmopolitan and several taxa present in only one site. This confirms the high sensibility of diatoms to local environmental conditions established elsewhere in mountain streams [61,106,109]. The cluster analysis also helped to confirm that communities from the two degraded sites differed the most from the other six sites, irrespective of the type of land use. The clustering also revealed that the variation in community composition among streams is equivalent to the variation in community composition within streams. This is, communities just a few hundred meters away from each other within the same stream were as different in their composition as communities from different streams and a few kilometers away. This may suggests that local processes (e.g., environmental filter, competition, grazing) are very important in determining diatom community composition in the tropical mountain streams around Loja. The local composition of diatom communities can be more influenced by the local habitat than by more general parameters of mountain streams physically altered by human activities [106]

Our study focused on studying the environmental and biotic characteristics of seven tropical mountain streams associated with different types of land use. Given the scarcity of studies on the community composition of periphyton in this type of streams, our results
may provide some unique information about the water condition of these poorly studied ecosystems. Some of our results show the effects that the degradation of the adjacent vegetation can have on the ecological status of streams and their water quality. First, the absence of forest directly leads to higher water temperature. Second, nutrient levels may also increase. Third, the increased temperature and nutrients seem to result in higher algal abundance. Fourth, the prevalence of cyanobacteria increased. Fifth, the number of taxa present in only one site decreased. Despite these changes, dissimilarity in community composition between zones of different land use within the same stream was equal to the dissimilarity in community composition amongst different streams. This suggest that diatom composition is determined by the sensibility of the different taxa to the local conditions, irrespective of the scale of spatial variation considered and despite the connectivity of the sites (within or among lakes). Two sites within the same stream but under very different physico-chemical conditions have the same impact on community composition as two sites from two different streams, irrespective of their environmental conditions. The degraded sites that showed clearer signs of degradation and thus had more different physico-chemical characteristics resulted in more dissimilar communities.
Supplementary material

**Figure S1:** map of *El Carmen* watershed representing land cover types (agricultural use in yellow and natural vegetation in green). Purple line represents the limits of the National Park *Podocarpus*. Pink dots represent sampling locations. Water drop-like symbol represents location of a water treatment plant.
Figure S2: map of Mónica watershed representing land cover types (agricultural use in yellow and natural vegetation in green). Purple line represents the limits of the National Park Podocarpus. Pink dots represent sampling locations.
Figure S3: map of San Simón watershed representing land cover types (agricultural use in yellow and natural vegetation in green). Purple line represents the limits of the National Park Podocarpus. Pink dots represent sampling locations.
Figure S4: map of Tenería watershed representing land cover types (agricultural use in yellow and natural vegetation in green). Pink dot represents sampling locations.
Figure S5: map of Las Violetas watershed representing land cover types (agricultural use in yellow and natural vegetation in green). Pink dot represents sampling location.
Figure S6: map of Volcán watershed representing land cover types (agricultural use in yellow and natural vegetation in green). Purple area represents the National Park Podocarpus. Pink dots represent sampling locations in each stream. Water drop-like blue symbol represent location of a water treatment plant.
Figure S7: map of Zhucos (or Shucos) watershed representing land cover types (agricultural use in yellow and natural vegetation in green). Pink dot represents sampling locations in each stream.
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CHAPTER VI

Main results and conclusions

This thesis explored two types of tropical high-altitude freshwater ecosystems located in Southern Ecuador: shallow paramo lakes in chapters II to IV and mountain streams in chapter V. Apart from describing some of their basic characteristics for the very first time, we used the information collected in the field to test three different aspects of the composition and functioning of ecological systems. First, the biodiversity and ecosystem functioning relationship. Second, the distance-decay hypothesis. Third, the impact of the degradation of the surrounding vegetation on stream water condition.

Main characteristics of lakes in Tres Lagunas

In general, the physical, chemical and biological variables estimated in Tres Lagunas are within the ranges of previous studies under similar geographic conditions in high-altitude mountain lakes from Ecuador. The lakes we studied are all below 10 meters deep. They are cold, non-stratified and acidic. The nutrient levels are very low, which defines the lakes as oligotrophic or ultra-oligotrophic. This suggests that the Tres Lagunas system has not yet suffered eutrophication despite the influencing presence of human activities. Probably due to the low nutrient levels and solar radiation that characterizes the páramos, the levels of chlorophyll-a concentrations are also very low. The phytoplankton diversity is also relatively low. Chlorophyll-a concentrations and conductivity were the two variables contributing the most to differentiate amongst lakes in Tres Lagunas. Diatoms are the dominant group of microalgae, followed by dinoflagellates. Green algae, cyanobacteria and golden algae were also abundant. We also found large spatial variation in the composition of phytoplankton communities. We hope this study will provide a baseline for future studies on the impact of climate change and human activities on tropical high-altitude freshwater lakes.
Biodiversity and ecosystem functioning in *Tres Lagunas*

We found that four abiotic factors combined explained 76% of the variation in chlorophyll-a concentration amongst lakes. Contrary to what studies from temperate regions suggest, taxa richness was not related to either chlorophyll-a concentrations or total phytoplankton biovolume. Moreover, phytoplankton biovolume diversity was negatively correlated to both chlorophyll-a concentrations and total phytoplankton biovolume. This was due to a very uneven distribution of productivity amongst taxa in the more productive lakes. To the best of our knowledge, this is the first attempt to explore the determinants of phytoplankton functioning in tropical high-altitude lakes. We hope that this study will help establishing a baseline for evaluating the consequences of human activities in the ecology and functioning of this vital but fragile ecosystems. Our results suggest that by modifying the abiotic and biotic parameters of tropical high-altitude lakes, human activities can indirectly impact their functioning and their capacity to provide vital ecosystem services.

Testing the distance-decay hypothesis in *Tres Lagunas*

Phytoplankton composition at the phyla level showed high among-lake spatial variation in *Tres Lagunas*. We tested the geographical and environmental components of the distance decay relationships in the phytoplankton structure. Our results revealed no evidence of a link between the geographic distance and phytoplankton composition. On the contrary, we observed some environmental related patterns of community structure. The absence of support for the distance decay relationship observed could result from a conjunction of local niche-based effects and dispersal limitations. Phytoplankton community composition in the *Tres Lagunas* system or any other ecosystem may be jointly regulated by niche-based and neutral forces that still need to be explored. Despite not proving a mechanistic explanation for the observed patterns of community structure, we hope our findings will help improving our comprehension on these vulnerable and vital ecosystems. More studies in tropical high-altitude lakes are urgently required.
Impact of the degradation of the surrounding vegetation on stream water condition

Compared to the areas with native vegetation along the stream, zones without pastures or crops showed an increment in total phosphates and temperature that probably lead to a higher algal abundance. These patterns might be indicators of the initial stages of a degradation of water condition in the cleared zones. We found that diatoms dominate the periphyton in the streams around Loja but also that there is a large spatial variation in community composition within and amongst streams. We observed an increase in the prevalence of cyanobacteria in the open zones, which represent a clear indicator of the degradation of water condition as a consequence of clearing the natural forest in the watershed. The overall diatom richness is low in these streams but almost half of them were rare and endemic to a zone in a stream. Whereas the average number of species in the open and forested zones were not different, the number of taxa present in a unique site was lower in the open zones than in the forested zones. Dissimilarity in community composition between zones of different land use within the same stream was equal to the dissimilarity in community composition amongst different streams. This suggest that diatom composition is determined by the sensibility of the different taxa to the local conditions, irrespective of the scale of spatial variation considered and despite the connectivity of the sites (within or among lakes). Two sites within the same stream but under very different physico-chemical conditions have the same similarity in community composition as two sites from two different streams, irrespective of their environmental conditions.
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