Universitat Autònoma de Barcelona

Microbial assemblage responses to nutrient fluctuations in high mountain lakes

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PhD Thesis 2019

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Nere guraso eta ahizpei

Helduidazu eskutik estu arren
ilun dago ta ez dakit bueltatzen
-BTX-

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CONTENTS

ABSTRACT

Cold, oligotrophic lakes hold a large diversity of planktonic microbial organisms, protists and prokaryotes. The coexistence of such a large variety of organisms and living forms has challenged the ecological theory. The environmental fluctuations, particularly the contrast between seasons, have been pointed as an opportunity for coexistence. As the bases of this thesis, it was hypothesized that episodic nutrient enrichments play a major role in the maintenance of such high microbial diversity, particularly because nutrient loadings throughout the year consist in different amounts and combinations of phosphorus and nitrogen compounds, providing an opportunity for nutrient niche differentiation.

Two approaches were used to investigate the planktonic microbial coexistence: field regular observations and experimental field manipulations. The first involved the monitoring of one annual cycle of the bacterioplankton community in a seasonally frozen lake and the second, an *in situ* nutrient enrichment experiment using enclosures. Both approaches were carried out in Lake Redon, an ultraoligotrophic deep high mountain lake that is ice-covered for about half a year. In Chapter 2, the seasonal changes of the bacterioplankton community are described. In Chapter 3 and 4, the protist- and bacteria-specific responses to episodic nutrient enrichments are investigated using self-filling enclosures and P and N additions.

A new paradigm for bacterioplankton seasonal changes in seasonally ice-covered lakes is suggested in Chapter 2. In contrast to the traditionally-assumed assemblage seasonal replacement, there is a core of bacterioplankton assemblages developing fundamentally during the ice-covered period, which is resistant or resilient to the high irradiances conditions of the summer season. A different assemblage develops in the epilimnion during the ice-free season for a short period, taking the opportunity of unsuitable conditions for the core assemblages that proliferate in most of the water column during most of the year.

The ENEX experiment aimed to investigate the relationship between episodic nutrient enrichments and species coexistence. Concerning the protist community (Chapter 3), P was the limiting nutrient promoting species competition, while N was involved in niche stabilizing mechanisms. Most of the species typically growing at different periods of the year were recovered during the experiment from epilimnetic waters, showing that, although at undetectable abundances, species have the capacity to persist for long periods in the water column and quickly recover from low densities when favourable nutrient conditions are present.

Concerning bacterioplankton (Chapter 4), there was also marked biomass increase to P enrichment. However, the comparison with the natural cycle in the lake showed that

most of the response was mediated by the phytoplankton growth and the higher availability of labile organic carbon. There was little segregation at high taxonomic ranks (i.e., classes) of the bacteria across nutrient conditions; however, OTUs from the same family segregated across the P gradient and N form sources indicating niche differentiation that can promote coexistence.

The thesis demonstrates the key role of episodic nutrient enrichments of varying composition for maintaining long-term stable non-equilibrium coexistence in the planktonic microbial community.

RESUM

Els estanys freds i oligotròfics presenten una elevada diversitat d'organismes en el plàncton microbià, tant de protists com de procariotes. La coexistència d'aquesta elevada diversitat d'organismes i de formes de vida ha canviat la teoria ecològica. Les fluctuacions ambientals, i particularment el contrast entre estacions, esdevenen una oportunitat per a la coexistència. D'acord a aquesta idea, s'ha hipotetitzat que els enriquiments de nutrients episòdics juguen un paper fonamental pel manteniment de l'elevada diversitat microbiana, especialment perquè les aportacions de nutrients al llarg de l'any consisteixen en quantitats variables de diferents compostos de fòsfor i nitrogen, proporcionant una oportunitat per a la diferenciació de nínxols.

S'han utilitzat dues aproximacions per a investigar la coexistència en el plàncton microbià: observacions regulars i manipulacions experimentals en el camp. La primera consisteix en el seguiment del bacterioplàncton al llarg d'un cicle anual en un llac que temporalment es congela i la segona, en un experiment *in situ* d'enriquiment de nutrients. Ambdues aproximacions s'han realitzat a l'Estany Redon, un estany d'alta muntanya ultraoligotròfic i profund que resta cobert per gel la meitat de l'any. En el Capítol 2, es descriuen els canvis estacionals observats en el bacterioplàncton. En els Capítols 3 i 4, s'investiguen les respostes específiques a l'enriquiment episòdic de nutrients en protists i bacteris, mitjançant tancats d'auto-ompliment i addicions de P i N.

En el Capítol 2 es planteja un nou paradigma per explicar els canvis estacionals del bacterioplàncton en els llacs que estacionalment es glacen. En contraposició amb el tradicionalment assumit que els conjunts d'espècies es van substituint estacionalment, hi va haver un nucli d'espècies que es va desenvolupar principalment durant el període cobert de gel que era resistent i resilient a les elevades radiacions del període d'estiu. Un conjunt diferenciat es va desenvolupar per poc temps a l'epilimnion durant el període lliure de gel, treien avantatge de les condicions poc favorables pel conjunt d'espècies que trobarem a la columna d'aigua del llac durant la major part de l'any.

L'experiment ENEX pretenia investigar la relació entre l'enriquiment episòdic de nutrients i la coexistència d'espècies. Pel que fa a la comunitat de protists (Capítol 3), el P va ser l'element limitant que va fomentar la competència entre espècies, mentre que el N va participar en mecanismes d'estabilització del nínxol. Moltes de les espècies que creixen en el llac en diferents moments de l'any es van recuperar durant l'experiment fet amb aigües epilimnètiques, demostrant que, encara que en abundàncies indetectables, les espècies tenen la capacitat de persistir en la columna d'aigua durant llargs períodes de temps i recuperar-se ràpidament des de les baixes densitats quan les condicions de nutrients els hi son favorables.

Pel que fa al bacterioplàncton (Capítol 4), també es va donar un increment de biomassa a l'enriquir amb P. No obstant, en comparar-ho amb el cicle anual natural del llac es demostra que la resposta venia sobretot donada pel creixement del fitoplàncton i la major disponibilitat de carboni orgànic dissolt. Hi va haver poca segregació dels bacteris entre condicions de nutrients a un nivell taxonòmic alt (classe); no obstant, OTUs propers es van segregar al llarg del gradient de P i fonts de N indicant una diferenciació dels nínxols que pot promoure la coexistència.

La tesi demostra el paper clau dels enriquiments episòdics de nutrients de composició variada per a mantenir una coexistència no-equilibrada estable a llarg termini en la comunitat planctònica microbiana.

Nothing in the life is to be feared
it is only to be understood

-Marie Curie-

CHAPTER 1
Introduction

General introduction

Microbial communities are characterized by the tiny size of the organisms that require the use of microscopic or molecular techniques to be approached. First attempts to address these communities date back to 1675 when Antonie van Leeuwenhoek described the "very little animalcules" (Barton & Northup 2011; Bertrand *et al.* 2015). However they were not introduced in the living world until 1866 when Ernst Haeckel proposed the three-kingdom scheme formed by Animalia, Plantae and Protista (Dayrat 2003; Kutschera 2016), and their huge diversity was not formally acknowledged until 1990 when the six kingdom system differentiated between protist, bacteria and archaea (Woese *et al.* 1990; Kutschera 2016).

Since the beginning, these small organisms caught the attention and curiosity of the scientific community; the interest on their different shapes, community dynamics and their relation to the environment encouraged scientists to plunge into this world. Nonetheless, due to their tiny size, it has always been a challenge to go through the world of microbial ecology. Diverse techniques have been developed in order to reach them, from the first optic microscopes set up by Hooke or Leeuwenhoek, to the molecular techniques that during the last decades have developed by leaps and bounds providing more detailed descriptions of microorganisms. Since 1980, the inclusion of next-generation sequencing (NGS) technologies has boosted the knowledge about microbial ecology owing to its potential to discern a wider spectre of microorganisms (Pedrós-Alió 2012). However, there is still a lot of work to be done, as new lineages are in constant discovery (Hug *et al.* 2016).

In aquatic ecosystems, first attempts to approach the minuscule planktonic community date back to the first half of the 19th century by the hand of Johaness Müller (Reynolds 2006). Nevertheless, the term "plankton" was not coined until the end of the 19th century by Victor Hensen; he thought that phytoplankton might provide food for animals in the sea like plant do provide for animals in the land. Anyhow, the minuscule sizes of these organisms lead to very numerous assemblages, where a large number of organisms belonging to different species coexist.

1.1 Abundance and diversity

Microorganisms account for a major fraction of Earth biosphere. Global biomass estimates suggest that microorganisms are the second-largest biomass component, and in marine environments might account up to 70% of the total biomass (Bar-On *et al.* 2018). In oligotrophic waters, estimates of the protist communities point to an amount

of 10^3-10^4 individuals per mL^{-1} (Reynolds 2006), and for prokaryotes, this value goes up to 10^6 cells per mL^{-1} (Cotner & Biddanda 2002).

Despite their tiny size, the role of microorganisms is highly relevant in the ecosystem, since their activity significantly affects the biogeochemical cycling of bioavailable elements (Limberger *et al.* 2017; Khachikyan *et al.* 2019). Microbial communities encompass a large phylogenetic diversity (Newton *et al.* 2011; Debroas *et al.* 2017), even in remote high mountain lakes (Liu *et al.* 2011; Kammerlander *et al.* 2015). This diversity has important implication for the stability and functioning of the ecosystem (Wittebolle *et al.* 2009). Only a few species are locally abundant (Pedrós-Alió 2012), the rest not growing, dormant species, or doing so extremely slowly compared with the abundant members (Pedrós-Alió 2006). The set of rare species have been referred as the rare biosphere (Sogin *et al.* 2006), which is considered highly relevant to confer long-term biogeochemical stability by providing functional redundancy and strong potential for compensatory dynamics (Caron & Countway 2009; Jousset *et al.* 2017). The abundance of rare species may rely on particular conditions and can spread out in favourable instances (Gardner *et al.* 2008; De Senerpont Domis *et al.* 2013; Shade *et al.* 2014; Vick-Majors *et al.* 2014; Burson *et al.* 2018). Distinct sets of microorganisms co-occur with apparently consistent temporal patterns (Pernthaler 2017). Although the inclusion of new technologies has allowed reaching previously undetected species, chances to obtain a realistic picture of the whole planktonic diversity are still methodologically limited; commonly only those more abundant species are identified (Pedrós-Alió 2012).

1.2 Environmental variation

Planktonic microorganisms have long been used as indicators of environmental changes because they respond rapidly to changes due to their short life-span (Adrian *et al.* 2009). Synchrony between seasonal changes and microbial plankton community composition has been repeatedly described (Sommer *et al.* 1986; Eiler *et al.* 2012; Piredda *et al.* 2016; Cruaud *et al.* 2019). Long-term studies have evidenced annually reoccurring successive community replacements as a consequence of the periodicity of seasonal changes (Berman *et al.* 1992; Crump *et al.* 2009; Fuhrman *et al.* 2015), although exactly the same patterns are hardly repeated, suggesting a non-equilibrium coexistence (Scheffer *et al.* 2003; Klausmeier 2010).

Although periods of significant environmental changes are part of Earth nature dynamics, a new era has arisen in which humans are the main drivers of global environmental change since the industrial revolution (Steffen *et al.* 2007). In this regard, the modifications of biogeochemical cycles are within the most marked risks affecting the Earth system stability (Steffen et al., 2015). Changes in biogeochemical cycles can

modify the seasonal episodic nutrient loads, one of the most influencing factors for microbial plankton communities (Power 1992; Carlson *et al.* 2007; Newton *et al.* 2011; Kaspari & Powers 2016; Burson *et al.* 2018) as nutrients play a relevant role as mechanisms controlling the coexistence of different species (Schoener 1974; Chesson 2000; Šimek *et al.* 2011).

Phosphorus (P) and nitrogen (N) are the main limiting nutrients in freshwater ecosystems (Schindler 1977; Smith 1982; Elser *et al.* 2007), and their fluctuations sustain high diversity of planktonic microbial communities (Sommer *et al.* 1986; Logue *et al.* 2012). Nonetheless, human activities have modified the biogeochemical cycles and unbalanced the N:P supply (Rockström *et al.* 2009; Steffen *et al.* 2015). The use of fertilizers in agriculture and industry processes are behind the increased emissions of N to the atmosphere (Galloway *et al.* 2008; Fowler *et al.* 2013), what has forced towards a P limited conditions (Goldman 1988; Peñuelas *et al.* 2013). Otherwise, atmospheric P has been associated to dusty regions (Camarero & Catalan 1996; Mahowald *et al.* 2008; Reche *et al.* 2009; Peter *et al.* 2014), but the rise of P rich compounds in the atmosphere during the last centuries is also consequence of the human activities (Peñuelas *et al.* 2013; Wang *et al.* 2015). Nutrients widespread hundreds of kilometres from the source and arrive even to remote areas driven by atmospheric circulation (Holland *et al.* 2005; Stohl 2006). Former studies have already reported evidence of N fertilization in remote and pristine areas (Bergström & Jansson 2006; Holtgrieve *et al.* 2011), and an increasing trend of P depositions during the last decades have been described in high mountain ranges (Camarero & Catalan 2012).

Unbalanced N and P lake inputs modify N:P stoichiometry provoking shifts on protists and bacterioplankton assemblages towards taxa favoured by the new N:P ratios (Elser *et al.* 2009; Hillebrand *et al.* 2013). P has been widely considered the main limiting factor in freshwater ecosystems, inducing the growth of the microbial plankton abundances when its availability increases (Schindler 1977; Carpenter *et al.* 1992; Brahney *et al.* 2015). But not all the species present a similar response to P additions, the tolerance to nutrient level and strategies to its acquisition varies among taxa (Grover *et al.* 1997; Sterner & Elser 2002; Klausmeier *et al.* 2004; Hillebrand *et al.* 2013). The different capacity to P uptake among taxonomical groups has been widely addressed (Phillips *et al.* 2013; Rofner *et al.* 2016), nonetheless it can be different even between phylogenetically closely-related species. Regarding the later, little is known about the species-specific responses to combined nutrient additions over phylogenetically or ecologically related phytoplankton species. But the gap of knowledge for bacterioplankton communities is even more pronounced, and ecological coherence of major taxonomic groups are commonly considered (Philippot *et al.* 2010).

N can also play a major role as limiting nutrient (Sterner & Elser 2008; Bergström *et al.* 2013). Oxidised nitrogen (NO_3^-) is usually the prevalent inorganic N form in lakes;

nonetheless, the proportion between NH_4^+ and NO_3^- change throughout the year. However, although the NH_4^+ is less abundant than NO_3^-, it is usually assumed that it is preferentially used by phytoplankton (Harrison *et al.* 1996; Glibert *et al.* 2016). The pressure among the selection of N form might have evolutionary resulted in a broad range of N uptake traits among phytoplankton (Litchman *et al.* 2007) and therefore might have entailed significant differences in phytoplankton and bacterioplankton composition (Canfield *et al.* 2010; Donald *et al.* 2013).

1.3 Case of study: high mountain oligotrophic lakes

Lakes, in general, are good sentinels of the climatic change because they can integrate changes in the surrounding areas and atmosphere (Adrian *et al.* 2009). Nonetheless, remote lakes, those occurring in high altitudes or latitudes, are even better sentinels since they have the advantage of not being overprinted by local anthropogenic processes (Catalan *et al.* 2013).

In high mountain lakes, similar to lakes from high altitudes, the strength of the seasonal variations entails the transition between very unlikely conditions, from cold and dark periods covered by ice (ice-covered season), to higher temperature and irradiance during the ice off period (ice-free season). Ice cover duration is an outstanding factor shaping microbial communities (Bertilsson *et al.* 2013; Hampton *et al.* 2017), and this period can be equal or even longer than the ice-off season in lakes from high latitudes or altitudes (Catalan 1989; Arp *et al.* 2013). Nonetheless, in the current climatic change scenario, the ice-covered season is expected to shorten, with earlier ice break-up (Šmejkalová *et al.* 2016) and intermittent winter ice cover (Sharma *et al.* 2009), what will greatly impact phytoplankton growth timing and lake primary production (Thackeray *et al.* 2008; Boetius *et al.* 2013; Beyene & Jain 2015).

Alpine lakes are generally oligotrophic (Catalan *et al.* 2009), and can portrait a comprehensive picture of the microbial plankton community shifts driven by atmospheric nutrient loads. First of all, because the oligotrophic conditions of the lakes cause a great dependence on the atmospheric deposition and inflow water (Catalan 2006; Camarero 2017; Ren *et al.* 2019). And secondly, because their remoteness prevents direct human impact on the catchment, keeping relative good pristine conditions (Catalan *et al.* 2013).

1.3.1 Seasonal variations of protist community

Ice-covered season

During the dark and cold period, the ice cover formation and snow accumulation restrict the atmospheric inputs and reduces the light penetration (Catalan 1989; Kirillin *et al.* 2012), thereby limiting the phytoplankton primary production (De Senerpont

Domis *et al.* 2013). Early studies of winter in high mountain lakes already indicated under-ice microbial activity (Felip *et al.* 1995; Felip *et al.* 1999b). Indeed, higher abundances of protist than previously expected have been quantified under-ice (Hampton *et al.* 2017) mainly due to occasional peaks of protist (flagellated chrysophytes and dinoflagellates) at the beginning of ice-covered period (Ventura *et al.* 2000; Katz *et al.* 2015), and successive growth of mixotrophic and heterotrophic flagellates, and ciliates (Felip *et al.* 1999b; Felip *et al.* 2002).

Ice-free season

The relevance of phytoplankton as primary producers focused the attention in the ice-free season because of the light availability and internal and external nutrient loads (Reynolds 2006). However, during this period, the physical conditions of the water column are more variable than under-ice, and changes in primary production are conditioned by the spatial and temporal heterogeneity.

Overturn periods, twice a year in dimictic lakes, are usually related to greater phytoplankton abundances due to internal nutrient loadings (Catalan *et al.* 2002; Pla-Rabes & Catalan 2011; Ding *et al.* 2017). Such conditions may encourage non-motile species adapted to nutrient inputs and higher turbulence, such as diatoms (Margalef 1978). Spring overturn comprises the transition between ice-covered and ice-free seasons. At this time, temperature and radiation increase, and the nutrients accumulated from the atmospheric deposition and catchment runoff enter through the water column providing sustenance to the planktonic communities (Catalan 1988; Catalan *et al.* 1992b; Cortés *et al.* 2017). This is one of the most variable periods of the year and is characterized by the emergence of phytoplankton species related to higher productivity (e.g., volvocales, diatoms) and large ciliates (Felip *et al.* 1999b; Felip *et al.* 2002). During autumn overturn, convective movements may facilitate the disturbance of nutrient-rich sediments and the increase of phytoplankton biomass by enhanced nutrient diffusion and transport (Berman *et al.* 1992; Felip *et al.* 1999a; Ventura *et al.* 2000). In this period, different algal blooms may take place, principally belonging to the groups of chlorophytes and diatoms (Reynolds 1980; Felip *et al.* 1999a; Tolotti *et al.* 2003).

During summer, the water column stratification can produce an unevenly nutrient distribution and, concurrently, epilimnetic exposition to high solar radiation as a consequence of high altitude location of the lakes, and the low extinction coefficients (Sommaruga 2001). Protist growth is reduced in the epilimnion, where large dinoflagellates dominate, and a deep chlorophyll maximum develops at the upper hypolimnion related to chrysophytes and cryptophytes increase (Felip & Catalan 2000; Mellard *et al.* 2011).

1.3.2 Seasonal variations of the prokaryotic community

Regarding prokaryotes, there are still only a few studies providing detailed data of temporal variations in high mountain lakes. Most of the studies focused the attention on epilimnetic water layers (Jones *et al.* 2012; Vila-Costa *et al.* 2013a; Ortiz-Álvarez *et al.* 2019), or on the transition between specific periods (Yannarell & Triplett 2005; Comte *et al.* 2006; Llorens-Marès *et al.* 2012; Aguayo *et al.* 2017). Although highly different microbial activities have been described during the winter period (Grzymski *et al.* 2012; Williams *et al.* 2012; Eronen-Rasimus *et al.* 2017; Denfeld *et al.* 2018), from heterotrophic bacteria (Bižić-Ionescu *et al.* 2014) to chemoautotrophs such as nitrifiers (Boyd *et al.* 2011; Christner *et al.* 2014), sulphur or iron oxidisers (Marteinsson *et al.* 2013; Achberger *et al.* 2016) and methanotrophs (Samad & Bertilsson 2017; Denfeld *et al.* 2018), the knowledge about under-ice dynamics is still biased (Powers & Hampton 2016). Bias may partially be caused by sampling difficulties during the winter period, but also because of the phytocentric view of plankton dynamics. Several studies have suggested a coupling between the protist and prokaryotic communities (Eiler *et al.* 2012; Rösel & Grossart 2012; Bižić-Ionescu *et al.* 2014), prevailing heterotrophic activities linked to phytoplankton production (Sarmento *et al.* 2016). However, it is not clear whether seasonal patterns are coherent for both communities, and hence, greater efforts should be invested in understanding the dynamics of bacterioplankton communities throughout the year.

1.4 Species coexistence

Species diversity of natural communities has long been a central topic in ecology (Margalef 1963); however, understanding the mechanisms behind the maintenance of species-rich communities is still a challenging issue (Record *et al.* 2014; Li & Chesson 2016). Concerned about a large number of planktonic species in apparently homogeneous conditions, Hutchinson proposed the paradox of plankton at mid-20th century (Hutchinson 1961). His work, based in the principle of competitive exclusion expected in situations of complete competitors (Hardin 1960), referred to a scenario where environmental fluctuations might hold conditions away from equilibrium allowing the persistence of different species at different times and therefore enabling species-rich communities. Since that, alternative theories have focused on this topic, proposing mechanisms that allow the non-equilibrium coexistence of species (Kemp & Mitsch 1979; Huisman & Weissing 1999; Huisman *et al.* 2001; Scheffer *et al.* 2003; Huisman *et al.* 2004; Kemp *et al.* 2005; Huang *et al.* 2016; Barraquand *et al.* 2018).

A stable coexistence has to be understood over a long time span. Densities of species do not have to show decaying long-term trends; if densities get low, they must not undergo an endless drift and should show a tendency to recover (Chesson 2000).

Considering species coexistence is about potential competitors, species of the same ecology or, in other words, the same guild (Chesson 2018). Species from the same guild can coexist when the stabilizing niche differences are large enough to counteract their relative fitness differences (Mayfield & Levine 2010; HilleRisLambers *et al.* 2012). The species with higher average fitness, better competitors, would displace species with a lower competitive ability (Chesson 2018), and the latter might be doomed to the exclusion if they did not present greater niche differentiation than the average in the community (Mayfield & Levine 2010; Gallego *et al.* 2019).

The base of the coexistence is that intraspecific competition exceeds interspecific competition, and thus, species tend to limit more their own populations rather than to compete with other species (Chesson 2000; Adler *et al.* 2007; Gallego *et al.* 2019). Coexistence likely results from high intragroup density-dependence (Barraquand *et al.* 2018), and when any species increases in abundance, the per capita growth rate decreases relative to other species slowing down the competitive exclusion (Adler *et al.* 2007). Low-density species can recover in the presence of species found in higher densities during fluctuations (Chesson 2000; Angert *et al.* 2009), or when their nutrient requirements are lower than the requirements of abundant species (Tilman 2011). In this regard, episodes of nutrient enrichments, related to seasonal fluctuations, can be the main factor involved in species niche stabilization providing differentiated conditions to species according to their requirement.

1.5 Combining observational and experimental approaches

Based on observational methods, alternative species densities response to episodic environmental fluctuations can be detected. But observational approaches fail in the attempts to focus on a unique factor since multiple factors fluctuate at once in nature (Thackeray *et al.* 2008). Hence, experimental approaches can help in distinguishing the effect of episodic nutrient loads from the simultaneously on-going environmental fluctuations (Hutchinson 1957).

One may expect that the response to an induced nutrient increase would be similar to the changes observed during episodic seasonal enrichments occurring at short-term. But designing suitable experiments may not be simple, particularly if dealing with communities from oligotrophic and cold lakes. Laboratory experiments with microbial plankton assemblages from oligotrophic lakes are usually rarely viable. Most species are difficult to maintain in cultures and light, temperature and system dimensions result rather artificial. Field experimental approaches are more promising and include from whole-lake manipulations to small-size containers (Fairchild *et al.* 1985; Deininger *et al.* 2017). The former are costly to perform and difficult to replicate, whereas the

latter may be useful for short incubations but too far from real-world for longer assessments.

1.6 ENEX experiment

The general goal of the ENEX experiment was to evaluate the effects of the fluctuations in P availability and N:P imbalance on the structure of the planktonic microbial community using pulse perturbations. It was based on a new design of enclosures fundamentally aimed to investigate the immediate response of the planktonic community, integrated across the photic zone, to pulse perturbations using before and after comparisons and multiple replication and treatments.

6.1 Enclosures design

The experiment was carried out in the ultraoligotrophic lake Redon located in the central Pyrenees (Box 1). The enclosures were conceived to integrate the epilimnetic water layer, thus consisted in 20 m long polythene bags (diameter: 8.5 cm) and two polyvinyl chloride (PVC) tubes, attached one at each extreme of the bag (Figure 1.2).

Box 1

Lake Redon is a high mountain oligotrophic lake located in the central Pyrenees (42º38'33" N, 0º36'13" E, 2232 m a.s.l.). It has an area of 24 ha and a maximum and mean depth of 73 m and 32 m, respectively, with a water average residence time of ~4 years (Catalan 1988). The catchment is relatively small (155 ha) compared to the lake area, with granodiorite bedrock and barely vegetated. The lake is dimictic and covered by ice during six-seven months. The photic zone (40-50 m) largely exceeds the seasonal thermocline (~15 m depth) during the summer stratification, and the epilimnion shows low chlorophyll levels compared to the upper hypolimnion (Felip & Catalan 2000). There is a large imbalance between P and N availability (Catalan 1992a) due to the high nitrogen deposition in the area (Camarero & Catalan 1996), although increased P atmospheric inputs have alleviated the difference (Camarero & Catalan 2012).

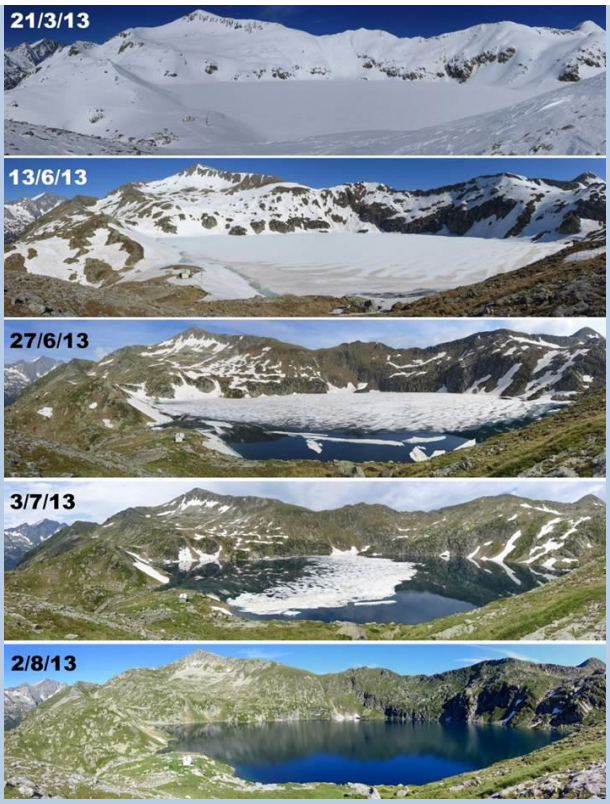

Figure 1.1 View of the seasonal variations Lake Redon between January and August 2013; comprising periods of winter inverse stratification, thawing and summer stratification.

The PVC tube at the lower end (length: 0.5 m) was closed and served as a sediment trap (Figure 1.2 D), while the upper tube (length: 1.5 m) enabled the gaseous exchange with the atmosphere, preventing the bag from collapsing (Figure 1.2 B). An expanded polystyrene float was attached to this upper tube to hold the enclosure at the water surface, and weight was tied at the sediment trap to stretch the bag. Each enclosure was self-filled with ~100 L of water from 0 to 20 m lake depths.

For deployment, the polythene bag was folded around the upper tube, and both tubes were held together by a rope lacing (Fig. 1.2 A). Then the enclosure was filled with the lake surface water and sank until 20 m depth, where the rope was tensioned and the lacing holding together both extreme tubes was untied. The upper tube floated up toward the lake surface, thus gently filling the bag with water from the upper 20 m of the water column (Fig. 1.2 A). Finally, extra floats were added to the upper part to increase the buoyancy (Fig. 1.2 B).

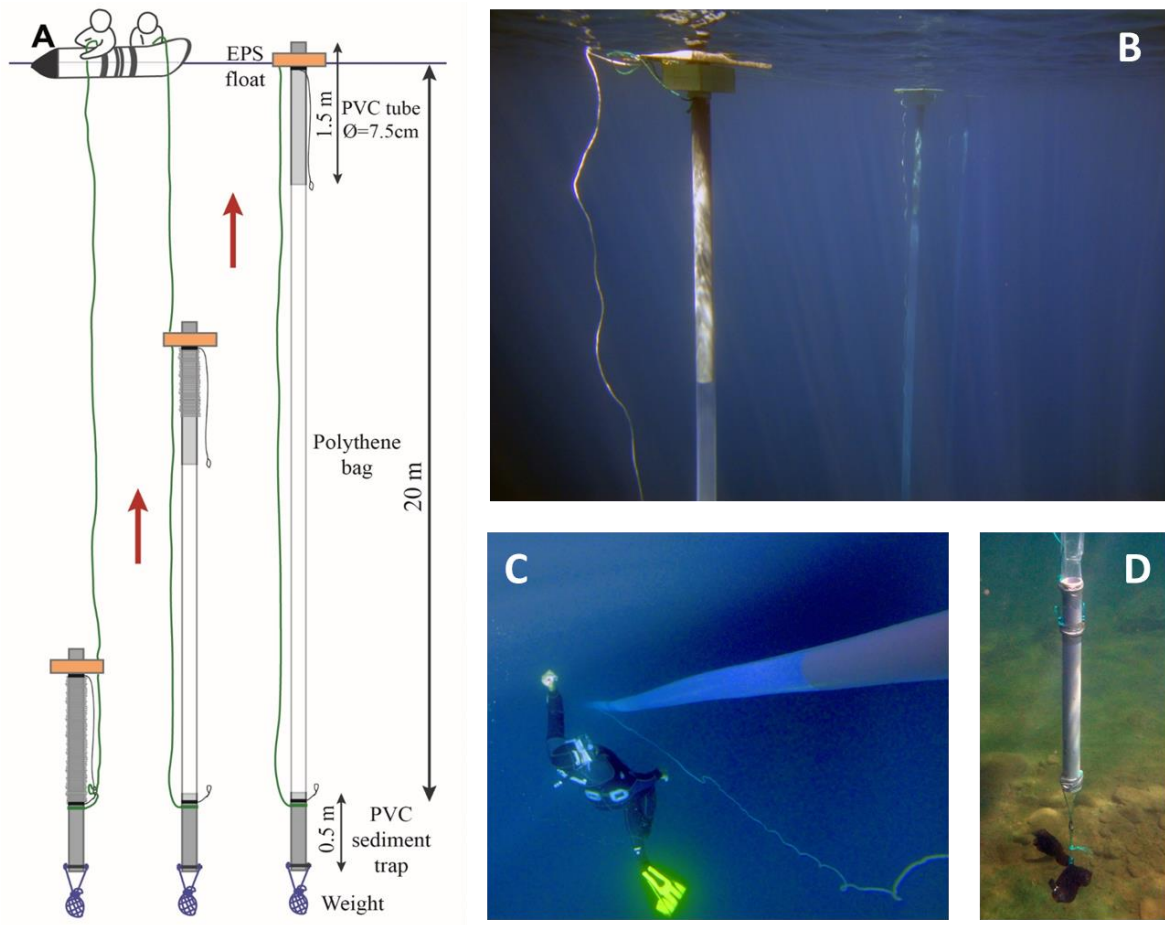

Figure 1.2. A) Enclosure sketch, deployment and filling procedure. Underwater view of the upper (**B**), middle (**C**) and bottom (**D**) parts of the enclosures.

Once the enclosure was filled, the specific additions of the treatments were performed using 20-m long thin plastic tubes, filling with ~0.9 L of nutrient-enriched water. The tubes were introduced inside the enclosure, and once the bottom was reached, the solution started to be released homogeneously along the enclosure's water column as the tube was withdrawn at a constant pace. Finally, all the enclosures were attached to a rope that held them to the enclosures mooring (Fig. 1.2 B).

At the end of the experiment, an integrated (0 to 20 m deep) water sample was obtained from each enclosure deepening 20-m long plastic tubes with the aid of weight. Five tubes (each ~1 L capacity) connected to a peristaltic pump deployed at the same time were used, and the sediment trap was recovered once the enclosure water column was sampled. The sediment trap was detached from the bag, and its water content was collected and kept until filtration in the laboratory.

6.2 Experimental design and considerations

Nutrient additions were designed taking into account the naturally occurring nutrient variation in Lake Redon. The ENEX experiment consisted in two nutrient gradients, one for P and other for N. P and N additions were designed to force the system into a less P depleted situation "P enrichment" and to an even more N unbalanced situation "N imbalance". Each nutrient enrichment gradient consisted of three concentrations: low, intermediate and high (Table 1.1), with the low-level treatment contributing to both P enrichment and N imbalance gradients. At each nutrient level, the dominant nitrogen source was either in the form of NO_3^- or NH_4^+ and, except in the controls, both P and N were added to each treatment to avoid that the addition of only one nutrient could induce the other to become limiting. N was added as NH_4Cl or KNO_3 and P as K_2HPO_4 and the control treatment (no additions) indicated the nutrient conditions in the lake water at the beginning of the experiment (Table 1.1).

All but the highest addition of P and N were within the seasonal variation range of TP, NO_3^- and NH_4^+ concentrations characteristic of oligotrophic lakes. The extreme enrichments provided mesotrophic conditions according to TP and N imbalance as a potential effect that atmospheric contamination may cause in lakes from remote regions.

Enclosures were deployed on 5-6 August 2013 and recovered 25 days later. Lake Redon is extremely oligotrophic systems, and due to the low populations in epilimnetic waters together with low-temperature conditions (14 to 6 ^oC), few weeks were a safety period for the experiment performance (Parsons 1982). The duration of the experiment was tailored to avoid collateral effects of long experiment durations such as a decay phase of the community or the transition to a secondary succession within the

enclosure. Nonetheless, the amount of material collected in the water column compared to that collected in the sediment trap was two orders of magnitude higher; suggesting the community still was in a growing phase (Giménez-Grau *et al.* submitted-b).

Table 1.1. Nutrient additions to obtain the P enrichment and N imbalance gradients both either with nitrate or ammonium as the main nitrogen source. The two gradients combined resulted in an N:P stoichiometric gradient. Each treatment was replicated in the experiment. The control values indicate the characteristics of the initial filling water.

GRADIENT	TREATMENT	TDP μmol L^{-1}	NO_3^- μmol L^{-1}	NH_4^+ μmol L^{-1}	DIN μmol L^{-1}	DIN:TDP (a/a)
Control	NA	0.022	4.2	0.2	4.4	200
P enrichment	NO_P++	1.9	16.8	0.2	17	9
P enrichment	NH_P++	1.9	4.2	12.8	17	9
P enrichment	NO_P+	0.21	16.8	0.2	17	81
P enrichment	NH_P+	0.21	4.2	12.8	17	81
P enrich/N imbal	NO_P	0.06	16.8	0.2	17	283
P enrich/N imbal	NH_P	0.06	4.2	12.8	17	283
N imbalance	NO+_P	0.06	34.8	0.2	35	583
N imbalance	NH+_P	0.06	4.2	30.8	35	583
N imbalance	NO++_P	0.06	72.8	0.2	73	1217

Another aspect considered in the experiment was the filling method. It was designed to minimize the stress of the organisms during the filling process and also to capture the vertical heterogeneity of the epilimnion. Enclosures that need for initial pumping or weighted integration of samples could contribute to initial bias, additionally can damage planktonic populations resulting in a community completely different from the initial pool. Conversely, the ENEX self-filling mechanism was friendly for organisms, and although could contribute to initial haphazard as the enclosures were not filled from a homogenous pool, the results were coherent to nutrient additions and replicates showed high similarity (Giménez-Grau *et al.* submitted-a).

The high aspect ratio of the enclosures (wall area vs water volume), together with the duration of the experiment and mesotrophic conditions in some treatments, increased the risk of benthic colonization at the walls. Such periphyton growth might condition the community assemblages in the water column and increase nutrient consumption. In order to determine the possible adverse effect of nutrients, bags were microscopically observed for algae, and some spots of filamentous algae (*Spirogyra*) were found, especially in P enrichment treatments. However, their development was not large enough to affect planktonic communities (Giménez-Grau *et al.* submitted-a).

Together with nutrients, grazing could have been an undesired source of variation between treatments. High grazing pressure and rapid nutrient regeneration maintain autotrophic biomass at low levels, and conversely, low grazing pressure relates to high autotrophic biomass (Hessen *et al.* 2013). The small diameter of the enclosure and the turbulence generated during the filling process should have partially excluded zooplankton that rejects the turbulence (Franks 2001). In the experiment, the total number of crustaceans was low. *Cyclops abyssorum* showed the lowest turbulence avoidance and were the most abundant in the experiment, followed by *Diaptomus cyaneus* and *Daphnia pulicaria* (Giménez-Grau *et al.* submitted-a).

1.7 Thesis objectives

The main goal of this thesis was to analyse the effect that seasonality and episodic nutrient loads had over bacterial and protist communities of cold, oligotrophic lakes. The observations and field experiments were performed in Lake Redon, where the seasonal variation of the protist assemblages was already known. Therefore, we studied the seasonal variation of the bacterioplankton community during an annual cycle and performed a nutrient enrichment experiment to evaluate the effects of the episodic nutrient availability on the structure of the planktonic microbial community.

In **Chapter 2**, the aim was to provide detailed information about the seasonal dynamics of bacterioplankton community in relation to the environmental fluctuations. In order to reduce the actual bias respect the ice-free season, the fieldwork consisted of a balanced sampling effort every 4 weeks during an entire annual cycle. The changes in bacterial abundance and diversity were based on molecular markers. We evaluated the resistance and resilience of the assemblages to the environmental fluctuations.

In **Chapter 3**, the aim was to unveil the specific response of planktonic protist species to nutrient enrichments. Based on inverted microscopy identification and counting, protist species abundances were estimated for the initial conditions of the lake and within the enclosures. Indicator species were established for the different treatments, and we analyse if they support the hypothesis that episodic nutrient enrichments stabilise the coexistence of protist species.

In **Chapter 4**, similar to the protist community, our purpose was to identify the bacterioplankton response to episodic nutrient enrichments. We first characterized the molecular composition of bacterial communities for all the enclosures and detected the indicator OTUs for each nutrient enrichment treatment. Afterwards, we related the nutrient indicator OTUs with their specific distribution across the lake seasonality studied in chapter 2 to discern between direct and indirect responses to nutrients.

In **Chapter 5**, we integrated the results obtained in the three previous chapters in order to visualize the contributions of this dissertation to the current knowledge of microbial plankton communities of cold, oligotrophic lakes.

CHAPTER 2
Resilience and resistance of under-ice bacterioplankton to the extreme seasonal changes in high mountain lakes

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2.1 ABSTRACT

Most high mountain lakes across the planet are covered by ice and snow for several months a year. A phytocentric view of the lake ecosystem could consider the ice-free period as the most relevant for the microbial lake communities. However, heterotrophic and chemoautotrophic prokaryotes could experience more favourable conditions under-ice. We used 16S rRNA gene sequencing to monthly monitor the bacterioplankton community of a deep high mountain lake in the Pyrenees. The results indicate richer bacterioplankton assemblages under the ice than during the ice-off period. The winter assemblages were resilient to the warm period persisting in the hypolimnion. On the other hand, a bacterial background assemblage, which was resistant to all depths and season conditions, was identified, mainly consisting of Actinobacteria ACK-M1. The summer epilimnetic waters, submitted to damaging high UV radiation, were the poorest in OTUs and showed a characteristic opportunistic assemblage. The bacterioplankton paradigm for long seasonally ice-covered lakes should consider the primary role of under-ice highly diverse resistant and resilient assemblages. Much reduced ice-cover periods due to global warming might drastically affect the bacterial communities of these lakes.

2.2 INTRODUCTION

Climate seasonality has a great influence on lake environmental conditions and affects planktonic communities (Diao *et al.* 2017; Morrison *et al.* 2017). In seasonally frozen lakes, coherence between bacterioplankton composition and seasonal changes such as ice cover, mixing and phytoplankton blooms have been described (Eiler *et al.* 2012). Due to the bacteria short lifetime and physiological diversity, the current paradigm assumes a replacement of communities when the conditions change across the seasons. Annually reoccurring patterns in bacterioplankton communities have been described in the oceans (Fuhrman *et al.* 2006) and rivers of polar areas (Crump *et al.* 2009; Cruaud *et al.* 2019). Conversely, the idea that prokaryotes are resistant and resilient is pervasive in microbial ecology, based on their metabolic flexibility and physiological tolerance to changing environmental conditions (Allison & Martiny 2008). These two views are not mutually exclusive in aquatic ecosystems. Rivers, small lakes and other systems with high water renewal may easily show replacement of communities. Higher water resident time in deep and large lakes might foster more complex and persistent communities, less affected by seasonal fluctuations (Lindström *et al.* 2005; Crump *et al.* 2007). Indeed, there is still rather limited empirical data on the seasonal variation of microbial communities across different types of aquatic ecosystems (Niño-García *et al.* 2016).

Lakes at high latitude or altitude, with a prolonged ice cover, constitute an interesting case. A long ice cover season should markedly influence the overall microbial composition and dynamics (Bertilsson *et al.* 2013; Hampton *et al.* 2017). However, it is not clear whether microbial eukaryotes and prokaryotes show similar seasonal patterns. Even little snow accumulation on top of the ice reduces the light penetration (Catalan 1989; Kirillin *et al.* 2012), largely affecting phytoplankton (Felip *et al.* 1999b), and mostly constricting their growth to the ice-free period (De Senerpont Domis *et al.* 2013). The view provided by phytoplankton dynamics is usually extrapolated to bacterioplankton because of the higher temperature of the ice-free period (Newton *et al.* 2011; Morrison *et al.* 2017) and the availability of phytoplankton exudates that provides fresh resources for bacterial growth (Hahn 2006; Bohórquez *et al.* 2017). Probably, this phytocentric view and the easier sampling conditions during summer have biased the studies of bacterioplankton communities towards the ice-free season (Bertilsson *et al.* 2013; Hampton *et al.* 2017). Even so, active and dynamic bacterial communities have been described during winter periods with different metabolisms (Williams *et al.* 2012; Eronen-Rasimus *et al.* 2017; Denfeld *et al.* 2018). From heterotrophic organisms degrading phytoplankton derived organic matter (Rösel & Grossart 2012; Bižić-Ionescu *et al.* 2014) to chemoautotrophic bacteria involved in different processes as nitrification (Boyd *et al.* 2011; Christner *et al.* 2014) or sulphur or iron oxidation (Marteinsson *et al.* 2013; Achberger *et al.* 2016). This large metabolic variety may let to high bacterial diversity during the under-ice period (Grzymski *et al.* 2012; Cruaud *et al.* 2019).

We studied the seasonal dynamics of the bacterioplankton community in a deep high-mountain lake, which is ice-covered by half of the year, using a balanced sampling effort between ice-on and ice-off periods. Based on the biogeochemical winter changes previously observed in this lake (Catalan 1992a), we expected a rich under-ice bacterioplankton community, which persistence during the ice-free period would be challenged by the extreme environmental differences between the two periods. We particularly focused on (i) how resistant or resilient those under-ice assemblages were to the seasonal changes and (ii) the degree of spatial and temporal coherence between phytoplankton growth and bacterioplankton community seasonal changes provided by the long duration of the ice-covered period.

2.3 MATERIAL AND METHODS

2.3.1 Site and sampling

The study was conducted in lake Redon, an ultraoligotrophic high mountain lake located at 2232 m a.s.l. in the Central Pyrenees (42º 38′ 33″ N, 0º 36′ 13″ E), with an area of 0.24 km^2, maximum and mean depth of 73 and 32 m, respectively, and water residence time of about 4 years . The lake is covered by ice and snow during 6-7 months a year (Box 1, Fig. 1.1) and stratifies during the ice-off season (Catalan 1988, 1989). Phytoplankton primary production usually peaks during the spring and autumn mixing periods, and a deep chlorophyll maximum develops during the stratification period when the photic zone extends beyond the seasonal thermocline (Felip & Catalan 2000).

Samples were collected every four weeks, from January 2013 to January 2014. Vertical temperature and O_2 profiles were performed at the deepest part of the lake at 1 m intervals using a 6920 V2 probe (YSI). During the ice-free season, water transparency was measured with a Secchi disk. Water samples were obtained at 2, 10, 20, 35 and 60 m depths and were filtered through a 250-μm pore size mesh to remove large zooplankton. A subsample for water chemical analysis was filtered through a precombusted (5h, 450 ºC) glass fibre filter (GF/F, Whatman), and the filter was used to analyse the particulate matter. Another water subsample (0.5 L) for chlorophyll-a (Chla) analysis was filtered in the same way and the filter wrapped in aluminium foil and frozen immediately to prevent degradation. Finally, a third subsample for 16S rRNA gene analysis was filtered through a 0.2 μm pore size polycarbonate filter (47 mm Ø) until saturation and preserved with lysis buffer (40 mM EDTA, 50 mM Tris, pH 8.3 and 0.75 M sucrose) at -80 ºC up to DNA extraction.

2.3.2 Chemical analyses

Water samples were carried to the lab in bottles without any air space to avoid any gas exchange that could change the *in situ* pH, which was measured with a fast response, low ionic strength electrode (Crison-Hach 5224). Total dissolved phosphorus (TDP), soluble reactive phosphorus (SRP), ammonia (NH_4^+) nitrite (NO_2^-) and dissolved reactive silica (DRSi) were determined by colourimetry using a segmented flow autoanalyser (AA3HR, Seal/Bran+Luebbe). For TDP and SRP, the method was based on (Murphy & Riley 1962) (Bran+Luebbe method G-175-96). For TDP, filtered samples were previously digested by the acid persulphate oxidation method (Grasshoff *et al.* 1983). NH_4^+ and NO_2^- were determined by the blue indophenol method (B+L G-171-96) and the Griess reaction (B+L G-173-96), respectively. DRSi was determined by molybdic-silicate reduction to heteropoly blue (B+L G-171-96). Nitrate (NO_3^-) and

sulphate (SO_4^{2-}) were measured by capillary electrophoresis (Quanta 4000, Waters). Dissolved inorganic nitrogen (DIN) was calculated as the sum of NO_3^-, NO_2^- and NH_4^+.Dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) were estimated by the difference between the measured total and the inorganic forms. Dissolved organic carbon (DOC) was estimated by catalytic combustion and infrared spectrometric detection (TOC-5000 Shimadzu). Particulate carbon and nitrogen (PC and PN) were determined using a Carlo Erba elemental analyser. Particulate phosphorus (PP) was determined by acid persulfate wet oxidation followed by SRP analysis. Chla was extracted in 90% acetone with an ultrasonic homogenizer (Sonopuls GM70 Delft, The Netherlands) (50W, 2 min); extracts were centrifuged (4 min at 3000 rpm, 4 °C) and filtered through a Whatman Anodisc 25 (0.1 μm), and quantified by spectrophotometry (Jeffrey & Humphrey 1975).

2.3.3 16S rRNA gene analysis

DNA was extracted using the phenol/chloroform method (Rusch *et al.* 2007) and quantified using Qbit fluorometer (Thermo Fisher Scientific Inc.). Quantitative real-time PCR (qPCR) was used to quantify 16S rRNA gene, in combination with the primers 341F and 534R (López-Gutiérrez *et al.* 2004) and iQ SYBR Green PCR Master Mix (Table S1). Replicates were conducted on different plates, and the average concentration per each sample was calculated.

Diversity and structure of the bacterial community were determined by the sequencing of the V3-V4 hypervariable region of the 16S rRNA gene. Amplicon libraries for each sample were generated using a two-step Polymerase Chain Reaction (PCR) protocol (Berry *et al.* 2011). The first step was carried out in duplicate per sample and consisted in 15 μL reaction blending 10 ng of the sample with 2X Phusion PCR Master Mix, 0.25 μM of each 16S rRNA universal primers Pro805R and Pro341F (Takahashi *et al.* 2014) and 0.5 $\mu g \cdot \mu L^{-1}$ BSA. Thermal cycling was performed for 25 cycles using the Applied Biosystems 2720 thermal cycler (Taula S2.1). Afterwards, replicates were pooled together and purified using the Agencourt AMPure kit. In the second step, 3 μL of the purified product were mixed with 0.2 μM of each Nextera adapter sequences and similar reagent concentrations as in the first step. This step was also performed in duplicate per each sample, consisting in 30 μL of reaction. The final products were pooled together and purified following AgencourtAMPure XP method and quantified using Qbit fluorometer (Thermo Fisher Scientific Inc.). Goodness of the product was visualized on 1.0 % SB gel before Illumina Miseq sequencing at Microsynth (Switzerland).

2.3.4 Sequence Data Analysis

Reads were trimmed, and quality-checked with FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and FastX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html), and pair-end reads were merged using PEAR (Zhang *et al.* 2013). Clustering was performed with vsearch (Rognes *et al.* 2016) with an initial dereplication step to eliminate all identical reads, and clustering at 97% identity. Chimeras were checked sequentially for de novo and with the 'gold' database as reference with the UCHIME algorithm implemented in vsearch. Finally, all clusters with size <5 were removed and reads mapped back at 97 % identity. The final OTU table was converted to BIOM format for downstream analyses. A third part of the reads was lost during the clustering process obtaining 3,956,971 mapped reads with 749 OTUs. The representative OTU sequences were classified against the non-redundant 16S rRNA SSU database in SILVA (v128) with SINA (Pruesse *et al.* 2012). After filtering for chloroplast and mitochondria removal, a table of 686 OTUs was obtained into philoseq object (McMurdie & Holmes 2013).

2.3.5 Numerical analyses

The spatial and seasonal distribution of the OTUs assemblages was studied by ordination with Principal Components Analysis (PCA) with the vegan R package (Dixon 2003; Oksanen *et al.* 2011) and K-means clustering (R Core Team 2014) in both cases using the Hellinger distance (Legendre & Gallagher 2001). The best K partition of the OTUs assemblages in clusters was established with the Total Indicator Value method (Catalan *et al.* 2009). The OTU Indicator Value (IndVal) (Dufrene & Legendre 1997) for a cluster was considered significant when $p < 0.05$ (De Cáceres & Legendre 2009) using the indicspecies R package (De Cáceres 2013). Diversity within each sample cluster was assessed using the Shannon index and significant differences between clusters were tested by ANOVA (999 permutations) and *post hoc* pairwise comparisons with HSD Tukey test (Oksanen *et al.* 2011; Chambers *et al.* 2017). Linear discriminant analysis (LDA) was performed to relate the OTUs assemblage clusters with environmental conditions (Tharwat *et al.* 2017). All the environmental variables were log-transformed and the MASS (Ripley *et al.* 2013), lda (Chang & Chang 2010) and flipMultivariates R packages used. Plotting was performed with ggplot2 (Wickham 2009), raster (Hijmans *et al.* 2015), fields (Nychka *et al.* 2018) and rworldmap (South 2011) R packages. All the analyses were performed with the R software version 3.4.3.

2.4 RESULTS

2.4.1 Seasonal environmental fluctuations

The lake is typically dimictic (Fig. 2.1). The ice-covered period extended until early July 2013 when the ice broke up, and the water column mixed in a few days. The stratification period covered from August to October and included a metalimnetic temperature gradient between 10 and 20 m depths (11 to 6 °C), and epilimnetic temperature always below 14 °C. The water column mixed again in November after a progressive thermocline deepening during September and October. At the end of November, the lake surface froze, and the inverted weak stratification at low temperature from top to bottom started again (0 to 3.8 °C).

The Chla and O_2 seasonal fluctuations summarize the main lake metabolism changes as indicators of phytoplankton growth and decay and the photosynthesis/respiration balance, respectively (Fig. 2.1). The highest Chla values occurred between 20 and 35 m depth in August and September. This deep chlorophyll maximum spread throughout the mixing upper layers when the thermocline was deepening during October. There was a slight increase in Chla at the beginning of the ice-covered period but, as soon as, heavy snowfalls began, Chla declined progressively throughout the under-ice period, becoming very low from March to June, especially in the deepest layers. The water column layers above 35 m depth were well oxygenated throughout the year, either because of the phytoplankton net production during the ice-free period or because of

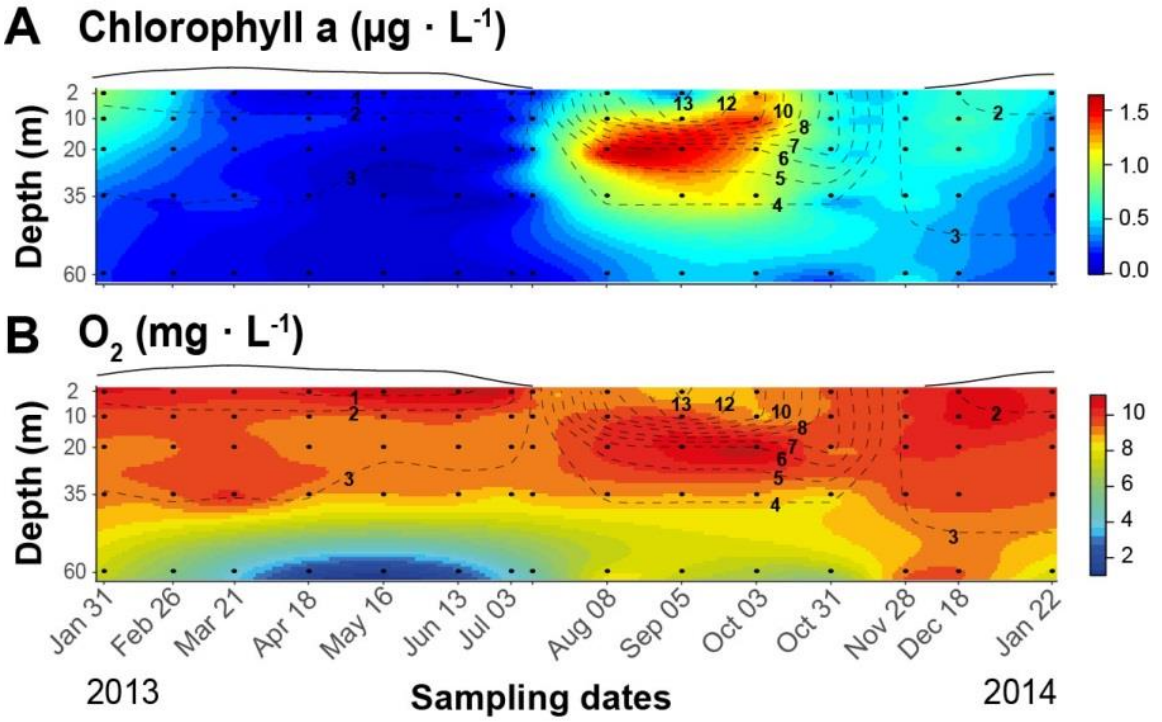


Figure 2.1. Seasonal and depth variation of chlorophyll-a (**A**) and oxygen (**B**) in Lake Redon, Pyrenees. Dots indicate sampling points and dashed lines the isotherms (°C). The upper outline indicates the ice and snow cover thickness in arbitrary units.

the low consumption and high solubility during the ice-covered period. In contrast, O_2 experienced higher fluctuations at deeper layers (>35 m). The O_2 concentration was similar to the upper layers during the autumn overturn and early ice-cover stratification, but much lower during stratification periods, reaching hypoxia values (<2 mg O_2 L^{-1}) at 60 m depth from April to June (Fig. 2.1 B).

2.4.2 Bacterioplankton and environment

The 16S rRNA gene abundance range was 1-14 x 10^5 copies per mL^{-1}, markedly increasing in the hypolimnetic layers during summer (Fig. 2.2 A). From surface to 20 m depth, the gene concentration was similar throughout the year with 2.6 x 10^5 ± 1.3 x 10^5 copies mL^{-1}. At 35 m depth, the gene abundance peaked to about three times the average values during the maximum in September. During summer, values were also higher at 60 m, about two-fold the average values. Considering the whole lake, the gene abundance per square meter peaked during the ice-free period (September, 19 x 10^{12} copies m^{-2}) driven by the hypolimnetic increase.

A total of 684 OTUs were distinguished belonging to 43 classes and 122 families with 137 OTUs of entirely unknown taxonomic position; a complete list is provided in Appendix 1. The classes including more OTUs were Betaproteobacteria (104 OTUs), Alphaproteobacteria (96 OTUs), Actinobacteria (55 OTUs), Gammaproteobacteria (44 OTUs) Saprospirae (37 OTUs) and Flavobacteria (27 OTUs).

The OTU richness from the surface to 20 m depth was similar in value and seasonal patterns. During the ice-covered periods, there were 300-350 OTUs, which peaked to about 400 during the two water column overturn periods; the number declined to about 250 during summer stratification (Fig. 2.2 B). At 35 m depth, the values and seasonal changes were similar, but without the decline during summer stratification, about 350 OTUs were maintained. This intermediate-depth was the one with less annual fluctuations (Fig. 2.2 B). The highest values were observed at 60 m depth, always >350 OTUs. The OTU richness homogenized over depths during both mixing periods at relatively high values (380-425 OTUs). Total OTUs richness across the whole water column was similar along the seasons (533 ± 31 OTUs).

The ordination of the OTU's composition using Hellinger-PCA analyses explained the 52 % of the variation in the two first axes (Fig. S2.1). The main axis captured the seasonal differences between under-ice and ice-free periods, and the vertical changes during summer stratification (August to October). The second axis was related to the differences between spring and autumn overturns. The combination of the two axes summarized the seasonal OTU's variation of the first 20 m of the water column in a wide oval trajectory, where temporal changes were more relevant than differences

between depths. However, the seasonal variation of the samples at 35 and 60 m was much lower than in the upper layers, occupying a centred position in the first axis, and moderately differentiated in the second.

The OTUs clearly segregated across the first axis, and some were closely associated with some periods of the season; for instance, OTU 2 (Flavobacteriaceae) with the summer epilimnetic samples, several OTUs with the under-ice period (e.g., 7 (Cerasicoccaceae), 3 (C111), 21 (Unknown), 17 (Gemmataceae), 9 (Actinobacteria) and36 (Betaproteobacteria)), 1 (Comamonadaceae) to the thaw period and 29 (Sphingobacteriaceae) to deep summer layers.

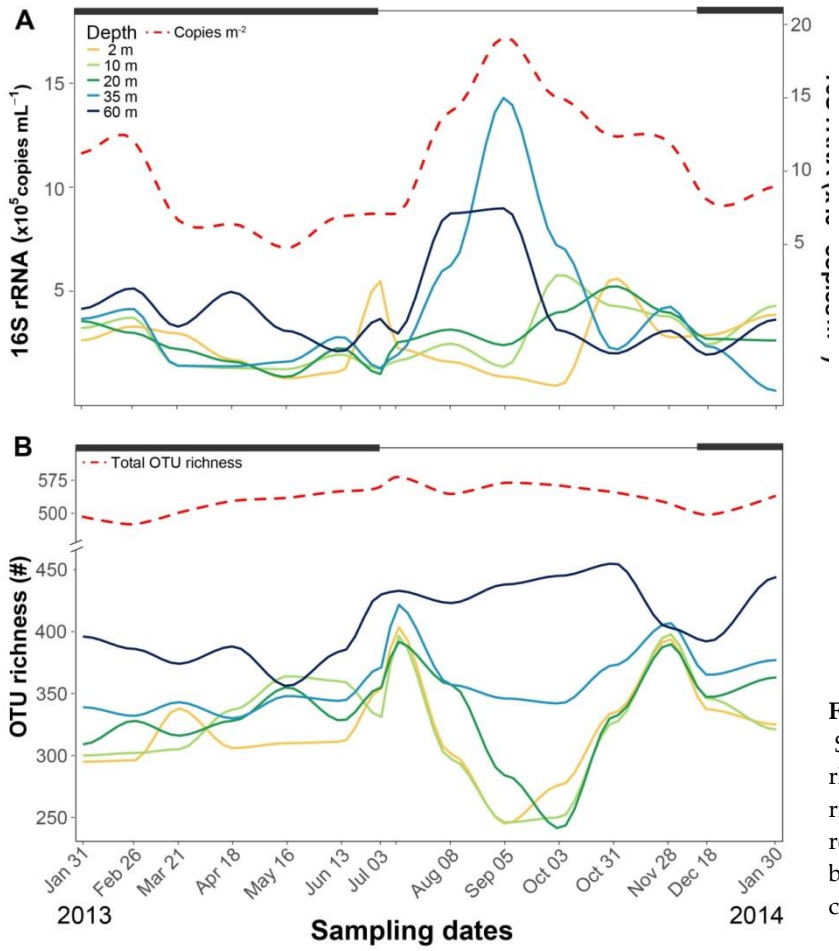


Figure 2.2.
Seasonal changes of 16S rRNA gene (A) and OTU richness (B) at five reference depths. Top black lines indicate ice cover presence

The seasonal and depth changes in bacterioplankton composition were classified using k-means clustering (Fig. 2.3 A). The Total Indicator Value showed the highest value when six groups were selected (Fig. 2.3 B), which hence was considered the best partition. There were 430 OTUs significantly related to one of the clusters and the rest (254), about one-third of the total, constitute a background assemblage with low fluctuations through time and space. The identified clusters reflected the main features of the seasonal change (Fig. 2.3 A). Thus we named them accordingly as "Under-Ice"

(UI), "Thaw and hypolimnion" (TH), "Early stratification" (ES), "Epilimnion" (EP) "Overturn and early under-ice" (OE) and "Deep layers" (DL). The UI cluster included the highest number of samples (28) from January 2013 to early July, including most of the depths, and a sample from October at 60 m depth. The TH cluster included the surface samples from April to July, all the depths during thaw water column mixing and most of the hypolimnetic samples during summer stratification. The ES cluster, with a few samples and short duration, corresponded to the early stratification period and comprised most of the depths except the 60 m layer. The EP cluster included the epi- and metalimnetic samples (0-20 m depths) for the rest of the stratification period, from early September to late October. Finally, the OE cluster of the autumn overturn and early under-ice, including from surface to deep layers. The DL cluster.

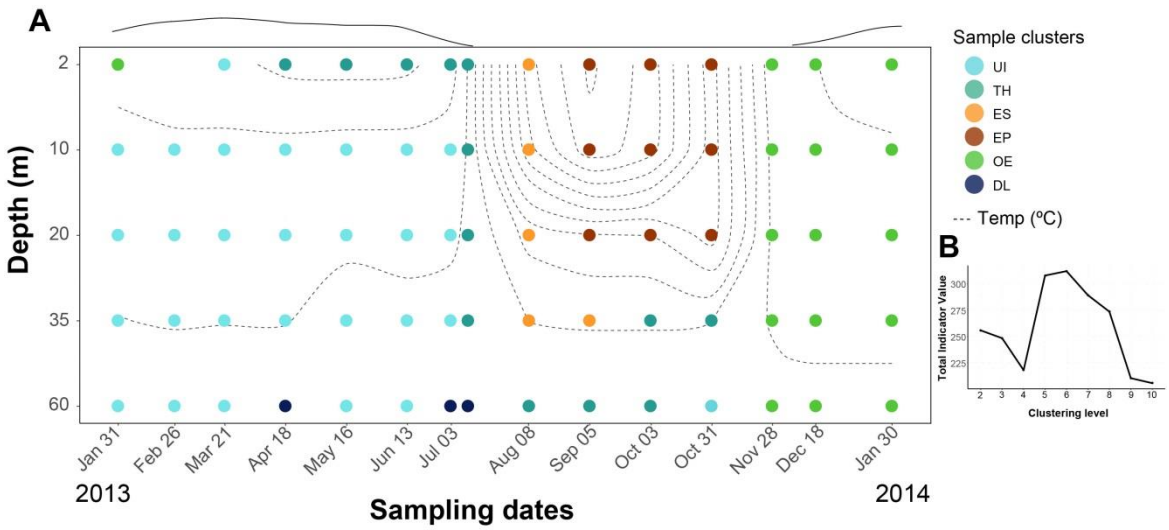
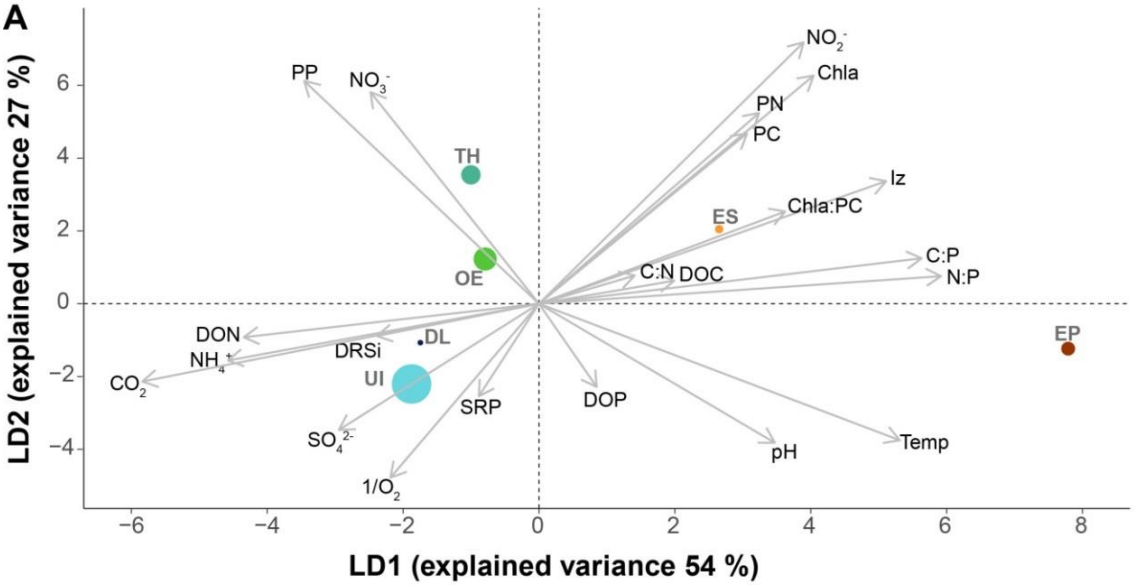


Figure 2.3. A) Distribution of the bacterioplankton groups obtained using k-means clustering with Hellinger distance. The best number of groups was assessed maximizing the total indicative value of the OTU that were significant at each partition (**B**). Dashed lines denote isotherms as a reference of the seasonal environmental changes; dots indicate sampling points and their colour the corresponding group: UI (under-ice), TH (thaw and hypolimnion), ES (early stratification), EP (epilimnion), OE (overturn and early under-ice) and DL (deep layer).

The environmental conditions associated with each bacterioplankton cluster were evaluated by Linear Discriminant Analysis (LDA). The main axis mostly differentiated between under-ice and ice-free conditions, whereas the second pointed to redox conditions (Fig. 2.4 A). The clusters obviously plotted accordingly to theses general seasonal and depth tendencies. In more detail (Fig. 2.4 B), there was a progressive transition of changing conditions across EP, ES, OE, TH, UI and DL. High values of N:P, C:P, Temp, and Chla at one extreme and high values of CO_2, NH_4^+, and DRSi at the other. Fundamentally, indicating conditions of P-limited primary production and conditions of deep-water heterotrophic recycling conditions, respectively.



B

	EP	ES	OE	TH	UI	DL
N:P	38.1	26.3	22.2	16.7	14.8	15
C:P	371	246	219	134	115	98
Temp (ºC)	8.8	6.2	2.5	2.3	2.8	3.5
Chla (µg·L^{-1})	0.84	1	0.45	0.24	0.12	0.05
Iz	0.63	0.42	0.13	0.21	0.001	0.03
pH	6.8	6.5	6.6	6.4	6.5	6.1
NO_2^- (µeq·L^{-1})	0.06	0.04	0.03	0.04	0.02	0.02
PN (µatg·L^{-1})	1.4	1.3	1.2	0.9	0.6	0.9
PC (µatg·L^{-1})	13.7	11.9	11.6	6.5	4.6	5.8
Chla:PC	0.07	0.09	0.04	0.04	0.03	0.01
O_2 (mg·L^{-1})	9.6	9.3	9.7	9	7.5	2.9
DOC (mg·L^{-1})	0.33	0.28	0.30	0.22	0.22	0.26
C:N*	10.1	9.3	9.8	7.8	7.9	6.4
DOP* (µatg·L^{-1})	0.1	0.1	0.07	0.05	0.7	0.003
SRP* (µatg·L^{-1})	0.009	0.01	0.009	0.008	0.01	0.009
SO_4^{2-} (µeq·L^{-1})	14.6	14.4	15	15.4	15.7	17.5
DON (µeq·L^{-1})	1.19	3.1	3.2	2.2	3	3.3
PP (µatg·L^{-1})	0.038	0.05	0.055	0.055	0.046	0.062
NO_3^- (µeq·L^{-1})	3.5	4.7	4	6.1	4.2	8.1
DRSi (µatg·L^{-1})	8.7	9.6	9.1	10.2	9.9	19
NH_4^+ (µeq·L^{-1})	0.6	0.41	1	1.8	1.5	4.1
CO_2 (µmol·L^{-1})	9	17.9	19	36.4	37.9	114

Sample cluster

Figure 2.4. A) Biplot of the bacterioplankton clusters in a discriminant environmental space. The symbol size is proportional to the number of samples included in the cluster. **B**) Heat map relating the environmental variables with the bacterioplankton assemblages. The variables are sorted according to their significance, with the lowest p-values at both extremes, and the less relevant variables in the middle. The (*) indicates no significant variables. The values within boxes indicate the average value of the environmental variables for the samples of the respective clusters.

2.4.3 Diversity in the seasonal assemblages

The number of OTUs with indicator value varied markedly from 31 in ES to 144 in DL. In general, the phylogenetic diversity in each cluster was high, as indicated by the number of taxonomic classes included among the respective indicator OTUs (Fig. 2.5 A).

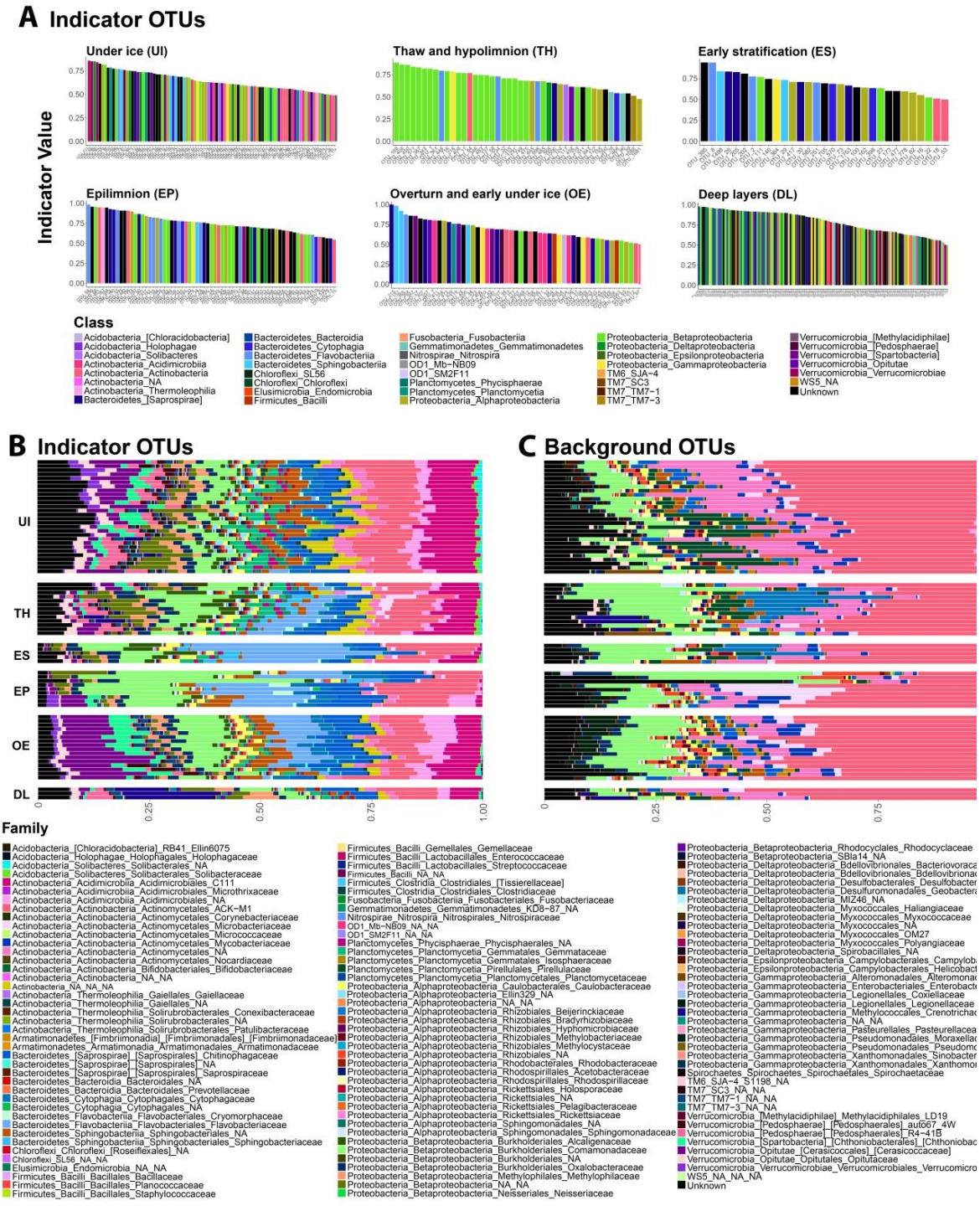


Figure 2.5. OTUs ranked for each seasonal cluster according to their significant indicator values. Colours indicate the taxonomic class to which they belong (**A**) and relative abundance of the Bacteria families in the samples considering indicator OTUs (**B**) and background OTUs (**C**). In **B** and **C**, samples are grouped and sorted by date and depth.

The exception was the TH cluster that showed a large proportion of Betaproteobacteria (mainly Oxalobacteriaceae and Comamonadaceae) as main indicators. DL was the most idiosyncratic cluster, despite the low number of samples included on it, it was the one with more indicator OTUs, which may reflect a connection with assemblages at the interface between the water column and the sediment. The overall assemblage diversity was lower in the epilimnetic clusters ES and EP (Fig. 2.5 B), particularly concerning indicator OTUs (Fig. S2.2 A). Interestingly, there were no diversity differences regarding background OTUs between clusters (Fig. S2.2 B).

In the UI cluster, the most abundant families were Verrucomicrobia (Cerasicoccaceae, Opitutaceae, Verrucomicrobiaceae, Chthoniobacteraceae and R4-41B), Actinobacteria ACK-M1, Acidimicrobia C111 and some unknown OTUs (Fig. 2.5 B). Following the seasonal sequence, the Flavobacteriaceae, Comamonadaceae, Alcaligenaceae and Oxalobacteriaceae gained relevance in the TH cluster assemblages, while the phylum Verrucomicrobia and Planctomycetes decreased markedly.

The bacterial assemblages strictly related to the summer stratification period, ES and EP, showed the lowest family richness (Fig. 2.5 B). The notable change was an increase in the abundance of Flavobacteriaceae, Chitinophagaceae and Comamonadaceae, as well as a significant decrease of the phyla Verrucromicrobia, Planktomicetes, Acidimicrobiia and unknown OTUs.

Following the summer stratification, the bacterioplankton community started to recover the composition found in the previous under-ice season (Fig. 2.5 B). A gradual decrease of the most abundant families in ES and EP samples, besides recovery of the families from UI assemblages, was characteristic of the OE samples. Verrucomicrobia was the most favoured phylum with the families Cerasicoccaceae, Chthoniobacteraceae and R4-41B, which started to increase at the end of summer but reached their higher abundances in OE samples. The Sphingobacteriales and Acidimicrobiia followed the same pattern than Verrucomicrobia. Bacteroidetes classes showed a particular indicator value for this cluster.

The idiosyncratic character of the DL cluster included Crenotrichaceae, Methylophilaceae and Actinobacteria (Fig. 2.5 B) and up to 29 taxonomical classes (Fig. 2.5 A). The OTUs with more indicator value included Bacteroidia, Crenotrichaceae, Desulfuromonadales, Chloroflexi, Endomicrobia, Fusobacteriia, Nitrospira, Mb-NB09 and SM2F11, Epsilonproteobacteria, SJA-4, SC3, TM7-1 and WS5.

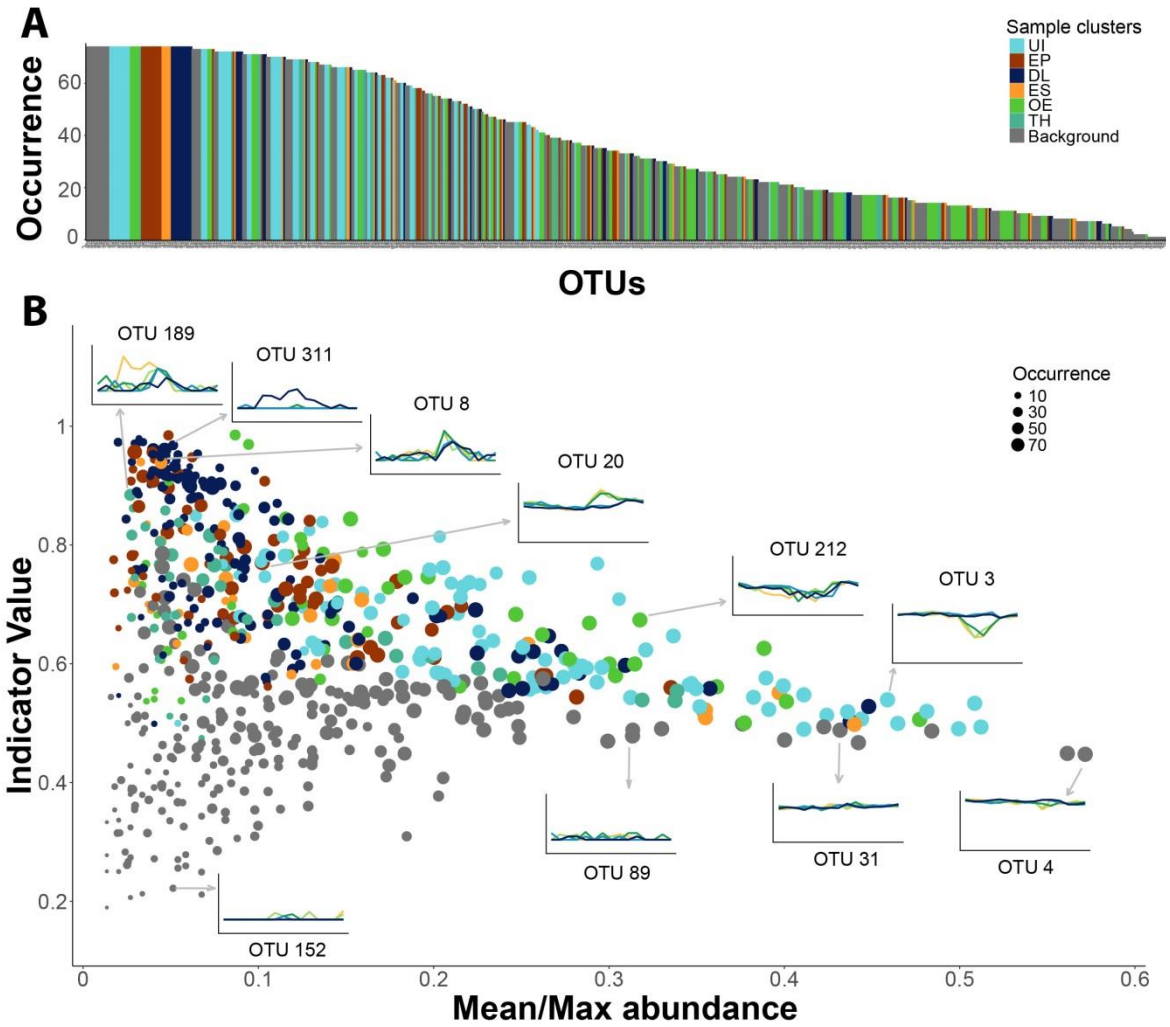


Figure 2.6. A) Rank of the OTU's occurrence in the samples distinguishing the respective indicator cluster. **B**) Comparison of the OTU indicator values with the ratio of the OTU mean and maximum abundance in the samples. The large the ratio, the less prompt to occasional blooming is the OTU. The symbol size is proportional to OTU's occurrence. The small line plots show some examples of the time series of the OTU abundance at each sampling depth (line colours as in Fig. 2.2). The arrows indicate the corresponding OTU in the larger plot.

2.4.4 The background assemblage

The OTUs that did not show any significant annual fluctuation were as common in the samples as those with indicator value (Fig. 2.6 A) and their relative sample abundance ranged from 9-33 %. In general, the lack of seasonal significance was due to the stability of the populations throughout the year and depths and not to low presence stochasticity (Fig. 2.6 B). Consequently, the composition of the background assemblages was quite similar throughout the year (Fig. 2.5 C). Some exceptions were rare OTUs (e.g. OTU 152 (Fig. 2.6 B), particularly of the families Microbacteriaceae and Cytophagaceae that appeared in the TH and EP samples, respectively. Actinobacteria were particularly abundant in the background assemblages, especially the family ACK-M1. Two of them presented a very stable time series, OTU 4 (Fig. 2.6 B) and OTU 41.

Unclassified Actinobacteria, Comamonadaceae and unknown OTUs were also abundant.

The time series of the background OTUs are a useful benchmark for comparing the patterns of the indicator OTUs. An OTU's indicator value was achieved in two different ways. On the one hand, the indicators related to UI and OE clusters had rather stable populations in most of the samples and declined in some specific conditions. For example, OTU 3 (Actinobacteria C111) and OTU 212 (Actinobacteria, Actinomycetales) showed similar abundances along the year but declined in the epilimnion during the ice-free season. On the other hand, the indicator value of OTUs in the ES, EP and TH clusters was related to their blooming during these specific periods. For example, OTU 20 (Betaproteobacteria, Comamonadaceae) indicator of the EP cluster, OTU 8 (Bacteroidetes, Flavobacteriaceae) of the ES, and OTU 189 (Betaproteobacteria) indicator of TH assemblage (Fig. 2.6 B). The indicator OTUs associated with the DL assemblage also may show blooming patterns but fundamentally show spatial segregation to the 60 m layer (e.g., OTU 311 Fig. 2.6 B).

2.5 DISCUSSION

The idea that microorganisms are resistant, resilient and functionally redundant is pervasive in ecology (Allison & Martiny 2008; Shade *et al.* 2011). However, there is still a limited number of aquatic ecosystems in which the persistence of microbial communities against the natural fluctuations had been studied with enough detail (Shade *et al.* 2012; Fuhrman *et al.* 2015; Kleinteich *et al.* 2017; Spears *et al.* 2017). The oligotrophy and the extreme seasonal changes characteristic of high mountain lakes constitute a compelling case to analyse the stability of the communities and the diversity of responses to seasonal fluctuations. From our results, we fundamentally may identify three types of the temporal dynamics of the OTUs. The background group, those OTUs without any particular period of enhanced growth or decline throughout the year, shows a resistant capacity in front of the highly fluctuating environmental conditions. High resilience is the feature of many OTUs that grew under low-radiation conditions, preferably under-ice but also in the hypolimnion, being penalized in the high radiation levels of the epilimnion but recovering quickly when these stressful conditions vanish. Finally, there are OTUs of an opportunistic character that grow exclusively in the epilimnion, where most of the other OTUs decline. An overall view of space and time variation of the bacterioplankton community identifies the summer stratification phase as a disturbance of the normal conditions in which a richer community preferentially develops. Even so, this episodic disturbance may fuel with organic carbon the bacterioplankton system as the much higher 16S rRNA number of copies in the summer hypolimnion may indicate.

2.5.1 The resistant assemblage

There were many OTUs that were not indicators of any of the seasonal clusters identified that, nonetheless, showed high abundance. Therefore, this assemblage must include organisms highly tolerant to the environmental fluctuations and physiologically flexible (Allison & Martiny 2008). Because of the lack of a seasonal preponderance, these organisms might call for less attention but actually represented a large fraction of the bacterioplankton abundance (9-33 %), including some of the most abundant OTUs. Interestingly, those OTUs belonged to the Actinobacteria ACK-M1 family from the acI lineage. Actinobacteria are ubiquitous in both terrestrial and aquatic ecosystems (Monard et al. 2016). In particular, the lineage acI is highly relevant in freshwater ecosystems (Warnecke et al. 2005; Newton et al. 2011), and the family ACK-M1 has been found in a wide range of ecosystems (Lindström et al. 2005; Eraqi et al. 2018). In the case of the highly oligotrophic and cold mountain lakes, there are some features of these taxa that may hold the key to the stability of their populations. On the one hand, Actinobacteria show a slower growth rate compared to other phyla (Newton et al. 2011) that may benefit them in oligotrophic conditions. Actinobacteria also show the capacity to store nutrients as polyphosphates (Forbes et al. 2009; Tarayre et al. 2016) and the ability to process N-rich compounds (Ghylin et al. 2014). If we consider the ice-free period conditions, several mechanisms of protection against high UV radiation have been described for Actinobacteria such as pigment production, strong cell-wall composition, high G+C content and DNA repairing capacity (Warnecke et al. 2005). Besides, the Actinobacteria acI lineage is endowed with actinorhodopsin (Sharma *et al.* 2009; Newton & McLellan 2015; Hamilton *et al.* 2017), which might allow using sunlight energy in a heterotrophic way (Dwulit-Smith *et al.* 2018) but also the capacity of producing complex carotenoids that could provide protection against high-radiation. Finally, their cell wall composition (Gram-positive) and the small cell size have been proposed as a refuge against grazing (Pernthaler 2005; Šimek *et al.* 2006); indeed, they are highly ignored by phagotrophic flagellates in mountain lakes (Ballen-Segura *et al.* 2017).

2.5.2 The resilient assemblage

The majority of OTUs identified showed better growth under low exposure to sun radiation. There was a connection in composition between the assemblages of the ice cover period, the spring, and autumn overturns and the deep hypolimnion during summer stratification. These assemblages showed particularly high diversity in winter as found previously under the ice in lake, and marine systems (Grzymski *et al.* 2012; Bertilsson *et al.* 2013; Marteinsson *et al.* 2013; Christner *et al.* 2014; Achberger *et al.* 2016). When the ice cover melted, the high family richness and OTUs abundance were

preserved, but their composition slightly changed. At this stage, there is a period of nutrient enrichment, both from the snowpack melting and water column deep mixing (Catalan 1992a; Cortés *et al.* 2017). Also, light penetrates the water column enhancing primary production, although temperature is still below 4ºC (Felip *et al.* 1999a; Salcher *et al.* 2008). The mixing spring period was short and the water column soon stratified. Due to the high water transparency in Lake Redon, the photic zone extends to the upper hypolimnion (~1 % of the surface irradiance reaches 50 m), so only the most profound layers remain into darkness and cold temperature across the year. The similarity in environmental conditions, therefore, connects the bacterial UI, TH and OE clusters. We can consider them as a resilient assemblage that is sensitive to the ice-free spring disturbance but quickly improves when the more common conditions are recovered (Allison & Martiny 2008). OTU 3 is a typical case; it was dominant during the ice-covered period, sensitive to the high-radiation period, and recovered quickly after the first snowfalls. The resilience of most of the under-ice assemblage was likely favoured by the depth of the lake that provides refuge against higher temperature and irradiance conditions. This finding is in agreement with another recent study of under-ice communities (Denfeld *et al.* 2018). In relatively shallow lakes, which are the most common in mountain lake districts (Mosquera *et al.* 2017), a high level of irradiance reaches the bottom of the lake, and thus the refuge is not present. The contrast between deep and shallow mountain lakes found in eukaryotic biota (Catalan *et al.* 2009) and sediment prokaryotes (Palacin-Lizarbe *et al.* 2019) may also extend to bacterial water column assemblages.

The most relevant OTUs of the under-ice assemblages belonged to the phylum Verrucomicrobia, which have been previously related to winter (Morrison et al. 2017; Cruaud et al. 2019) and oligotrophic conditions (Kolmonen et al. 2011). In Lake Baikal, they were also found under-ice associated with degradation of phytoplankton blooms (Bashenkhaeva et al. 2015). More specifically, the Verrucomicrobiaceae and Chthoniobacteraceae, which have been related to degradation of organic matter under-ice (Tran et al. 2018) and extracellular polymeric substances (Bohórquez et al. 2017), were notably relevant after the autumn overturn and beginning of ice cover in our study, period following the last seasonal phytoplankton peaks (Felip et al. 1999a). Other lineages indicators of the ice-covered were Planctomycetes (Strous et al. 1999; Tal et al. 2006) and a wide diversity of Acidobacteria (Fierer et al. 2007; Männistö et al. 2013). Noteworthy, some of the OTUs more abundant and characteristic of this period could not be assigned to any Bacteria phylum. The high phylogenetic diversity likely holds a large functional variety only partially explored (Auguet *et al.* 2012; Vila-Costa *et al.* 2013b).

The thawing period and deep hypolimnion resulted favourably to Betaproteobacteria, especially to Comamonadaceae, Oxalobacteriaceae and unclassified Burkholderiales.

Betaproteobacteria is one of the most abundant and diverse lineages in freshwater ecosystems (Newton et al. 2011), common in both oligotrophic and eutrophic ecosystems (Lindström et al. 2005; Šimek et al. 2006; Fierer et al. 2007; Newton & McLellan 2015). Betaproteobacteria were apparently favoured by the increase in nutrients during thaw and spring mixing, which will be in agreement with previous studies (Hahn 2006; Salcher et al. 2008). Probably, this connection was mediated by the increase of phytoplankton growth, in particular, Cryptophytes and Chlorophytes and their extracellular production (Šimek et al. 2011). In lake Redon, both groups are usually found in deep layers (Felip et al. 1999a) forming a deep chlorophyll maximum in the hypolimnion. The genus Polaromonas, known to be mainly psychrotrophic (Willems et al. 1991; Raymond-Bouchard et al. 2018) and one of the most abundant taxa in glaciers (Willems 2014; Gawor et al. 2016), was especially significant in this period among the Betaproteobacteria.

The bacterial composition of the deep layer (DL) cluster was highly distinctive and characterized by many indicator OTUs not present in other samples. In fact, the lake is 73 m deep, but the shape of the lake basin changes at about 60 m depth, markedly increasing the sediment proportion in contact with the water volume and changing the water chemical composition (Catalan 1988). These bottom lake conditions were not planned to be sampled, but it may happen that occasionally the 60 m samples may be influenced by them, as it seems occurred in late winter. The deep layers are deficient in oxygen and enriched in CO_2, NH_4^+, NO_3^-, SO_4^{2-} , among other compounds, thus providing substrates for a large variety of metabolisms. For instance, we found *Geobacter* and Actinomycetales with the capacity for CO oxidation (Llorens-Marès *et al.* 2015); the methanotrophs *Crenothrix*, Methylacidiphilales and Beijerinckiaceae (DeLong *et al.* 2014; Knief 2015; Samad & Bertilsson 2017); the methylotrophs Methylophilaceae, *Methylobacterium* (DeLong *et al.* 2014; Ricão Canelhas *et al.* 2016) and Rhodocyclacea (Smalley *et al.* 2015); Helicobacteraceae (Epsilonbacteria) able of anaerobic carbon fixation (Arnon cycle) (Llorens-Marès *et al.* 2015); nitrifiers as the commamox Nitrospira (Daims *et al.* 2015) and Burkholderiaceae (Peura *et al.* 2015); denitrifiers such as *Crenothrrix* (Oswald *et al.* 2017), and Helicobacteraceae (Han *et al.* 2012); dissimilatory nitrate reducers to ammonium, some Bacteroidales (Llorens-Marès *et al.* 2015); organisms able of nitrogen fixation as Endomicrobia (Zheng *et al.* 2016); sulphur oxidation OTUs as Helicobacteraceae (Kodama & Watanabe 2004; Han *et al.* 2012); sulphite reducers Desulfobacteraceae (Almstrand *et al.* 2016), OD1(Wrighton *et al.* 2012) and *Geobacter* (Fleming *et al.* 2006; Bravo *et al.* 2018); Fe (III) reducers as *Geobacter*, *Geothrix* (Lovley *et al.* 2011) and TM7-1 (Winsley *et al.* 2014) and Hg methylators as Desulfobacteraceae, *Geobacter* and Myxococcaceae (Gilmour *et al.* 2013; Parks *et al.* 2013; Bravo *et al.* 2018). OTUs with reduced genomes due to their parasitic lifestyle were also prevalent in the DL cluster, as the TM6 (Delafont *et al.* 2015; Yeoh *et al.* 2015), TM7-3 (Winsley *et al.* 2014) and Rickettsiales (McCutcheon & Moran 2012). All this diversity

indicates a microbiome in the deepest layers of the lake quite differentiated from the main water volume.

2.5.3 The opportunistic assemblage

The epilimnetic conditions were harmful to many OTUs, but some of them found the conditions to proliferate. In the current context of the lake, they can be seen as part of an opportunistic assemblage within the whole bacterioplankton community (Jousset *et al.* 2017; Pernthaler 2017). These organisms had the capacity to increase in harsh conditions, high UV irradiance (Catalan 2006), a temperature much above the usual values across the year, organic matter of poor quality (high C:P and N:P ratios). Although the OTU richness was low in these conditions, 16S rRNA abundance did not differ from the under-ice values at 2, 10 or 20 m depths; thus indicating that some OTUs took advantage of the situation. The adverse effects of UV on bacterioplankton have been broadly investigated including experiments in high mountain lakes (Herndl *et al.* 1993; Halac *et al.* 1997; Sommaruga *et al.* 1999; Sommaruga 2001; Santos *et al.* 2012; Sarmento *et al.* 2015). However, it is also known that sunlight can stimulate the growth of specific bacterial taxa resistant to high UV exposure. Precisely, some Bacteroidetes OTUs were among the best indicators of ES and EP cluster phylum of this period. A phylum with members highly resistant to UV radiation (Alonso-Sáez et al. 2006), and also able to grow in media enriched in carbon and poor in nutrients (Fierer et al. 2007). In any case, the reasons for success did not need to be the same for the different groups of Bacteroidetes present (Saprospirae (Chitinophagaceae), *Flavobacterium*, Sphingobacteriaceae); they may include resistance to grazing, opportunistic fast growth related to phytoplankton blooms and preference for higher temperatures (Pernthaler *et al.* 2004; Pernthaler 2005; Salcher *et al.* 2013; Neuenschwander *et al.* 2015).

The highest abundances of Betaproteobacteria in the epilimnion were related to the genus *Limnohabitans*, which is known to show a tight relationship to algal exudates and thus frequently has been related to productive periods (Pérez & Sommaruga 2006; Jezbera *et al.* 2012; Šimek *et al.* 2013). Alphaproteobacteria were present with contrasting living forms: Caulobacteraceae, with capacity for stalked cell formation and likely grazing resistance (Newton et al. 2011), Rhizobiales, with capacity for atmospheric nitrogen fixation (Newton et al. 2011) and Rhickettsiales with parasitic lifestyle (McCutcheon & Moran 2012).

2.5.4 A new view on high mountain lake bacterioplankton dynamics

Early studies of winter high mountain lake dynamics (Catalan 1992a) and ice and snow cover biological activity (Felip *et al.* 1995; Felip *et al.* 1999b) already indicated that

under-ice microbial activity, despite the low temperature (0-3 ^{o}C) was remarkable. However, insights into the bacterioplankton composition were technically limited. When the current molecular methods have been available, knowledge has increased, although there is much to investigate if we consider that a fifth of the sequences in our study was not classified at any taxonomical rank. In general, the focus has been placed mostly on the ice-free period (Bertilsson *et al.* 2013; Ortiz-Álvarez *et al.* 2019); partially, because of the difficulty of sampling during winter, but also due to the phytocentric view of plankton dynamics. The need to consider under-ice dynamics for a balanced understanding of all kind of lakes that experience a freezing period has been recently stressed (Hampton *et al.* 2017). Our study provides a balanced sampling effort throughout the year in a lake with half a year ice cover and shows that the winter period rather than epilimnetic summer waters constitute the fundamental period determining many of the features of the bacterioplankton community. We suggest as a new paradigm that there is a core of bacterioplankton assemblages fundamentally developing at low irradiance — either during the ice cover period or deep in the hypolimnion — that is resistant or resilient to the high irradiance summer conditions. Added to this core, there are opportunistic assemblages that bloom at specific periods of the season, being the most singular those that preferentially grow in the harsh conditions of the epilimnetic water.

Our results showed that the under-ice assemblages were the most characteristic OTUs pool of the lake's bacterioplankton instead of the one of the ice-free season, as it was previously assumed since the blooms of phytoplankton promote the growth of heterotrophic bacteria (Sarmento *et al.* 2016; Bohórquez *et al.* 2017). Summer stratification is a period of profound vertical gradients. The epilimnion assemblages are impoverished in diversity compared to the rest of the year but show a highly idiosyncratic composition. Deep waters show the compositional link to the bacterioplankton assemblages of the rest of the year and also the highest abundance of bacterioplankton. Therefore, in terms of productivity, bacterioplankton is linked to the phytoplankton dynamics, yet not in diversification and community complexity, which is higher under the ice even in the apparently homogeneous environment of the top layers.

Seasonal changes in bacterioplankton are often seen as a succession of communities that replace each other (Fuhrman *et al.* 2006; Crump *et al.* 2009; Eiler *et al.* 2012). Our results indicate a more stable community with an ample core of OTUs that are resistant or resilient to the seasonal changes. This feature may be related to the relatively long water residence time in Lake Redon (~4 years) and its depth (73 m). In shallower lakes, washout and lack of summer refuge may imply a rather different bacterioplankton dynamics, where recolonization dynamics may be more relevant and thus interannual variability more likely. Another aspect to consider — either comparing lakes or dealing

with global warming impacts— is the duration of the ice cover. In the case studied, ice-free and ice-covered periods are quite similar in duration. In this situation, it appears that the core bacterioplankton assemblage is determined by the winter conditions. During the months of the highest solar radiation (May, June, July), the ice and snow cover still protects the bacterioplankton community. If the duration is shorter, which is an on-going global trend (Sharma *et al.* 2019), it may jeopardize the current resilience of these under-ice rich assemblages. Likely, a sudden shift to a much different characteristic community may happen, and the opportunistic assemblage particularly adapted to the actual epilimnetic conditions may gain overall relevance. We suggest that ecological thresholds related to lake size and ice cover duration may characterize regime shift in the bacterioplankton communities of high mountain lakes.

2.6 SUPPORTING INFORMATION

Taula S2.1: Primers and thermal cycling details for the 16S rRNA qPCR and Illumina sequencing.

Target	Genes Primer names	Sequences (5'-3')	Thermal cycling	Technique	References
Bacteria 16S rRNA	**16S rRNA** 341F 534R	 CCT ACG GGA GGC AGC AG ATT ACC GCG GCT GCT GGC A	(95°C, 7min)x1 (95°C, 15s; 60°C 30s; 72°C, 30s; 80°C, 10s)x35 (95°C, 15s;(60 to 95°C, 10s increment 0.5°))x1	qPCR	López-Gutierrez *et al.*, 2004
Prokaryote 16S rRNA	**16S rRNA** Pro341F Pro805R	 AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGC-TCTTCCGATCTCCTACGGGAGGCAGCAG**CCTACGGGNBGCASCAG** CAAGCAGAAGACGGCATACGAGATNNNNNNGTGACTGGAGTTCA-GACGTGTGCTCTTCCGATCT**GACTACNVGGGTATCTAATCC**	(98°C, 3 min)x1 (98°C,30s; 55°C,, 30s; 72°C, 45s)x8 (72°C, 5min)x1	PCR for NGS	Takahashi *et al.*, 2014

Figure S2.1: Ordination (PCA-Hellinger) of the bacterioplankton community composition between January 2013 to January 2014 at 5 sampled depths 2, 10, 20, 35 and 60 m.

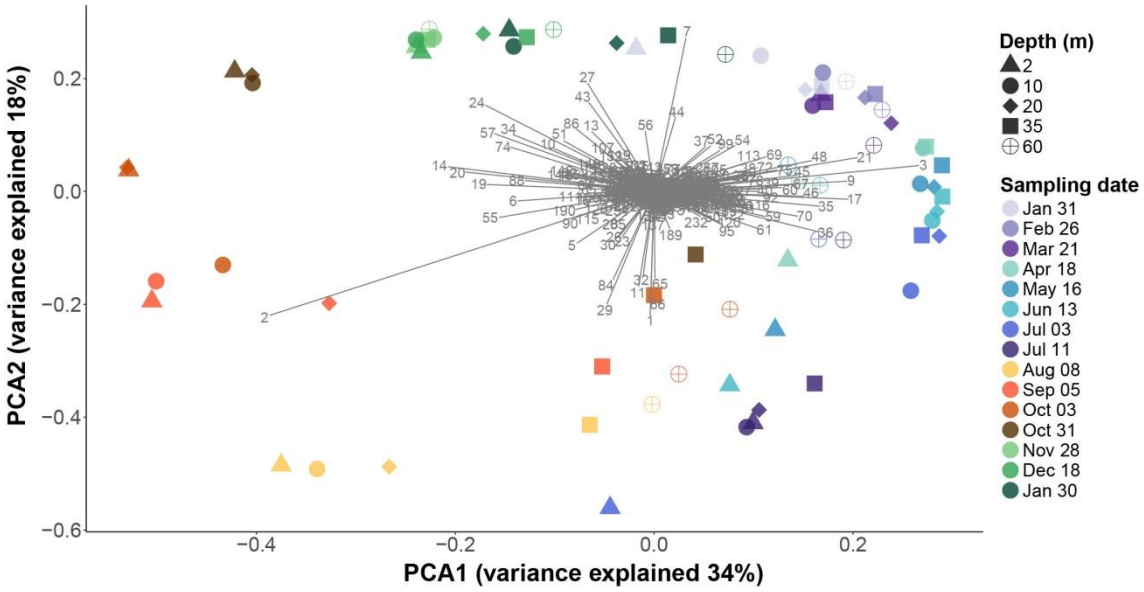
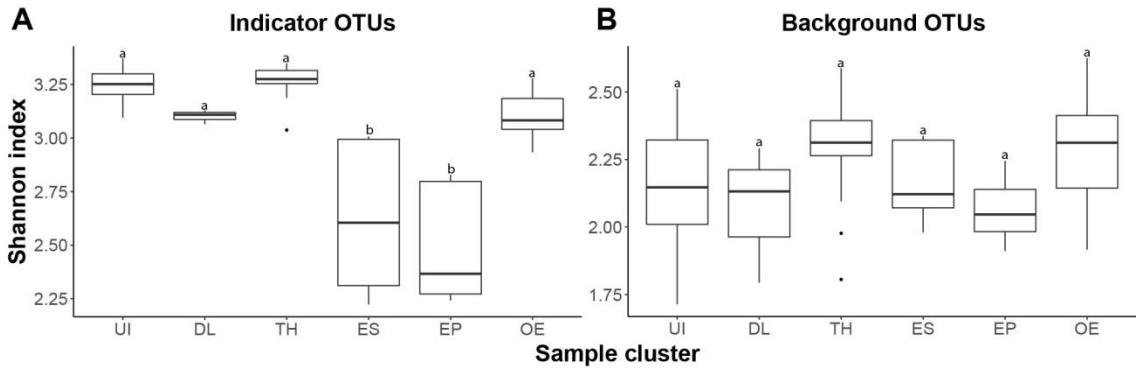


Figure S2.2: Boxplot of the Shannon index of each sample group considering the Indicator OTUs (A) and the Background OTUs (B).



CHAPTER 3
Episodic nutrient enrichments stabilise the species coexistence of highly diverse planktonic protist communities

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3.1 ABSTRACT

A wide variety of theoretical models provide explanations to the apparent paradox of the high diversity of planktonic communities. However, linking field observations to theory becomes elusive. We experimentally perturbed the apparently species-poor epilimnetic planktonic assemblage of an ultraoligotrophic P-limited lake using self-filling enclosures with nutrient pulse additions. Most protist species recorded in previous lake seasonal studies were recovered. Phylogenetically close species clustered across levels of phosphorus availability, but niche differentiation mostly occurred according to the type of nitrogen supply, including heterotrophic and autotrophic organisms. Autotrophic species with low initial density showed higher maximum growth rates than initially abundant species with similar nutrient niches. Fitness and niche differentiation proxies showed an inverse relationship. The results indicate that the lake protist assemblage could be considered a stable community of species that alternately recover from low densities with episodic nutrient enrichments and maintain long-term coexistence. Storage effects may play a central role.

3.2 INTRODUCTION

The high richness of species shown by planktonic communities in contrast with the apparent homogeneous conditions of their environment was highlighted by Hutchinson (1961) as the paradox of the plankton. This issue is a general case in ecology because similar situations are found in other types of ecosystems, e.g., desert vegetation (Waide *et al.* 1999), grasslands (Chu & Adler 2015), and tropical forests (Connell 1978; Wiegand *et al.* 2007). The apparent paradox was motivated by the theoretical competitive exclusion expected in situations of complete competitors (Hardin 1960). Since Hutchinson's paper, alternative theories and conjectures have provided explanations for the plankton paradox based mostly on theoretical and modelling insights (Kemp & Mitsch 1979; Huisman & Weissing 1999; Scheuring *et al.* 2000; Huisman *et al.* 2001; Schippers *et al.* 2001; Scheffer *et al.* 2003; Huisman *et al.* 2004; Shoresh *et al.* 2008; Cropp & Norbury 2012; Kenitz *et al.* 2013; Menden-Deuer & Rowlett 2014; Record *et al.* 2014; Huang *et al.* 2016; Cuesta *et al.* 2018) and some lab experiments (Sommer 1984) and field data analyses (Stomp *et al.* 2007; Keitt 2008; Downing *et al.* 2014; Alexander *et al.* 2015; Barraquand *et al.* 2018). Not all mechanisms proposed are mutually exclusive, and many could be stated as variants of theories on non-equilibrium coexistence in temporally fluctuating environments (Loreau 2010). The necessary condition is that all species should show positive long-term average growth rates; therefore, any negative difference in average fitness (competitive ability) between two species has to be compensated by stabilising growth components related to niche differentiation (Chesson 2018).

Plankton communities usually show marked changes in dominant species at relatively short time scales (e.g., seasonally in lakes). The community could be seen as successive transient situations in which species are replaced because of changing physical, nutrient and grazing conditions. Mechanisms driving gross seasonal biomass patterns of phyto- and zooplankton are well-understood, but species replacement requires more attention (Sommer *et al.* 2012). Implicit in the conception of species replacement, there is the assumption of immigration and local extinction dynamics. In the case of protists, however, this view might be conditioned by the limited empirical capacity to penetrate in the actual diversity existing at any time in the planktonic communities (Smetacek 2018). Even in the case of molecular techniques, the sequencing depth is still limited to account for the total diversity of planktonic microbial communities (Pedrós-Alió 2012). The number of individuals in these communities is extremely high, even not considering prokaryotes. In oligotrophic lakes and the open ocean, there are ~10^3-10^4 protist cells mL^{-1} (Reynolds 2006) — more than a billion in a cubic meter — ~10^{16} in the photic zone of a lake. Considering these extremely large numbers, we can hypothesise that stable non-equilibrium coexistence mechanisms may maintain a rich planktonic protist community that can only be partially observed at any particular time. Episodic environmental fluctuations will favour alternative species to achieve densities that make them detectable to our observational methods.

From field time series, it would not be easy to conclusively disentangle which changes are mainly driven by nutrient inputs and which by other co-occurring environmental shifts (Thackeray *et al.* 2008). Improving our knowledge of the role of episodic nutrient enrichments on planktonic protist assemblages requires complementing observations with experimental approaches. We may conjecture that if a water volume sufficiently large were confined experimentally to prevent immigration and submitted to different nutrient pulse perturbations, within the range naturally occurring, a large part of unseen species of the coexisting community should be recovered. This is the experiment we performed. During the ENEX experiment, we used enclosures to submit the planktonic community of a P-limited ultraoligotrophic lake to pulse nutrient additions to study the protist response.

We still lack a wealth of empirical detail about the direct and higher-order interaction between planktonic protist species and also a theoretical framework to properly evaluate coexistence. Nonetheless, in the framework of contemporary coexistence theory (HilleRisLambers *et al.* 2012), we postulated several heuristic pieces of evidence that together may support that episodic nutrient enrichments stabilise the coexistence of protist species . (1) In a P-limited ecosystem, relative fitness (i.e., maximum growth rates) differences should be related to P availability; whereas stabilising niche differences should be related to the variation in growth rates under changing nutrient combinations. Consequently, at least for autotroph, we should expect a negative

relationship between respective proxies of relative fitness and niche differentiation. (2) Frequency-dependent growth should be present if stabilising mechanisms compensate for fitness inequalities in a coexisting framework (Adler *et al.* 2007). In our case, for instance, species maximum growth rates should show a negative correlation with the relative species density at the beginning of the experiment. (3) Stabilising niche differences should also appear as a trait/phylogenetic clustering along the P axis (Mayfield & Levine 2010). That is, the main phylogenetic groups of primary producers should peak at different P levels. (4) On the other hand, differential responses to ammonium or nitrate N sources or N imbalance under similar P availability will reveal stabilising niche differences, particularly, if there is a trait/phylogenetic overdispersion of the species. (5) Summer epilimnetic waters in an ultraoligotrophic lake usually show very low protist biomass and diversity values. If the latter is an apparent feature of the technical difficulties in penetrating the low-density populations of the coexisting community, we should recover with the experimental treatments many species usually observed during other lake seasons and depths. Likely, treatments simulating more infrequent environmental conditions should recover rarer species.

3.3 MATERIALS AND METHODS

3.3.1 Experiment description

The ENEX experiment aimed to test the role of episodic nutrient enrichments in the species coexistence of protist species. The experiment was performed in Lake Redon (Fig. 1.2, Box 1), an ultraoligotrophic deep high-mountain lake (Ventura *et al.* 2000). We performed pulse nutrient perturbations of the summer epilimnetic protist community using enclosures of a volume (i.e., 100 L) in which we could expect 10^8-10^9 protist individuals. The planktonic community in the lake was P-limited (Camarero & Catalan 2012). We modified the P availability, the N imbalance respect P, and the N source (ammonium vs nitrate) to provide a range of conditions to evaluate the competitive ability across the limiting resource (P) and potential niche differentiation across stoichiometric variation and N supply.

The enclosures consisted of self-filling 20 m-long transparent cylindrical bags closed by a sediment trap, which were vertically deployed, and in which nutrient additions were performed. Enclosure construction, deployment, manipulation and performance are detailed in Giménez-Grau *et al.* (submitted-a). We performed the experiment at the middle of the lake stratification period when the epilimnion and metalimnion show the lowest chlorophyll concentration (Felip & Catalan 2000), and thus growth of the species can proceed with less competition when limiting nutrients are added. In natural conditions, episodic N increases may correspond to nitrate and ammonium.

Consequently, all the treatments were replicated using alternatively one of these N forms. Eleven treatments were performed with two replicates; thus, 22 enclosures were deployed (Fig. 1.2). The total phosphorus, nitrate and ammonium levels selected were within the oligotrophic seasonal and interannual variation found in the lake and snow cover, plus some extreme treatments above this range, providing mesotrophic conditions according to TP and nitrogen imbalance levels of regions highly affected by N atmospheric contamination. Both P and N were added to each treatment to avoid that the addition of only one nutrient could induce the other to become markedly limiting at some point during the experiment. We established a gradient of P additions ("P enrichment") and another of N ("N imbalance") (Table 1.1). Two enclosures with no nutrient addition (NA) were used as a reference for the enclosure effect. Unfortunately, one replicate of the NO_P++ treatment was lost during the field experiment. The enclosures were deployed and treated on 5-6 August 2013 and recovered 25 days later. The prolonged incubation was due to the ultraoligotrophic character of the lake and cold waters (6-14 ^{o}C) in the epilimnion. Two integrated water samples of the epilimnion and upper metalimnion, from 0-20 m depth, were collected on 6 August 2013 to assess the initial conditions of the experiment.–The protist abundance was evaluated microscopically, and the biovolume was estimated adjusting appropriate geometrical forms to the species shape (Hillebrand *et al.* 1999). See Supp. S3.1 for details on sample collection and analysis.

3.3.2 Numerical methods

Growth rate for each species (i) and treatment (j) were calculated as $r_{ij} = (\ln (B_{ij}/B_{io})/t$, where B indicates the species biovolume at the end of the experiment (j) or the average value at the initial water column conditions (o), and t is the experiment duration. Biovolume (biomass) was used instead of density because we assumed competition for resources (Li & Chesson 2016). Species that were not detected in the initial water column samples were assumed to have an initial density at least one order of magnitude lower than the minimum density measured (other assumptions did not change the main results). As a proxy for relative fitness (RF), we used the maximum growth rate found for a species in any of the treatments. The average correlation of the growth rate of a species with the rates of the rest, multiplied by -1, was used as a proxy for niche differentiation (ND). In both cases, for comparison, the values were standardised to z-scores. General calculations and statistics were performed in R version 3.4.3.

The protist community response to the nutrient treatments was evaluated using multivariate regression trees (MRT) (De'ath 2002) with the Hellinger distance (Cieslak *et al.* 2012). The method consists of a binary site clustering by repeated splitting, with

each split defined by a simple rule based on environmental values, in our case nutrient additions. This rule is chosen to maximise the homogeneity of the data and to minimise the impurity (total sum of squares of the response variable values to the node mean), and each cluster (or node) represents a group of associated habitat defined by the species assemblage and the environmental values. Experimental (21) and initial (2) samples were included in the analysis. The best MRT was selected using the minimal cross-validation relative error (MCVRE) criteria (Breiman *et al.* 1984; Tibshirani & Tibshirani 2009) and 999-permutation tests. The package mvpart in R version 3.4.3 was used (De'ath 2002; De'ath 2007).

The MRT provided a hierarchical clustering of response to the different treatments. We used the IndVal index (Dufrene & Legendre 1997) to determine the species characteristic of each cluster at several split levels. The procedure compares species fidelity and specificity to groups against randomised species distributions among them. We used the R package indicspecies (De Caceres *et al.* 2012). A 999-permutation test was used with no site combination (duleg=TRUE) and the association function "IndVal.g". Only species with p-value < 0.05 were selected as indicators of the groups. The indicator species of the same cluster can be considered as sharing a similar nutrient niche (i.e., N, P).

3.4 RESULTS

3.4.1 Species response to nutrient enrichments

Considering the experimental enclosures and the two integrated samples to assess the initial composition, a total of 151 protist taxa were distinguished: 88 were autotroph species, 42 heterotrophs and 21 were cysts that could not be related to any particular species (Fig. 3.1 A), except the *Dinobryon cylindricum* and *Uroglena* sp. cysts. Some species, not possible to be determined in preserved samples, were amalgamated into genera by size, particularly in the case of soft chrysophytes (e.g., *Ochromonas* spp (~7 μm), *Chromulina* spp (5 μm)), and a few into higher taxonomic levels. The number of taxa distinguished in each enclosure was between 60 and 90, similar to or higher than in the initial samples (50-60). About 39% of the taxa were detected in the two initial water column samples. Less than 20% of the taxa were found in only one enclosure, and about 50 % appeared at least in half of the enclosures at the end of the experiment (Fig. 3.1).

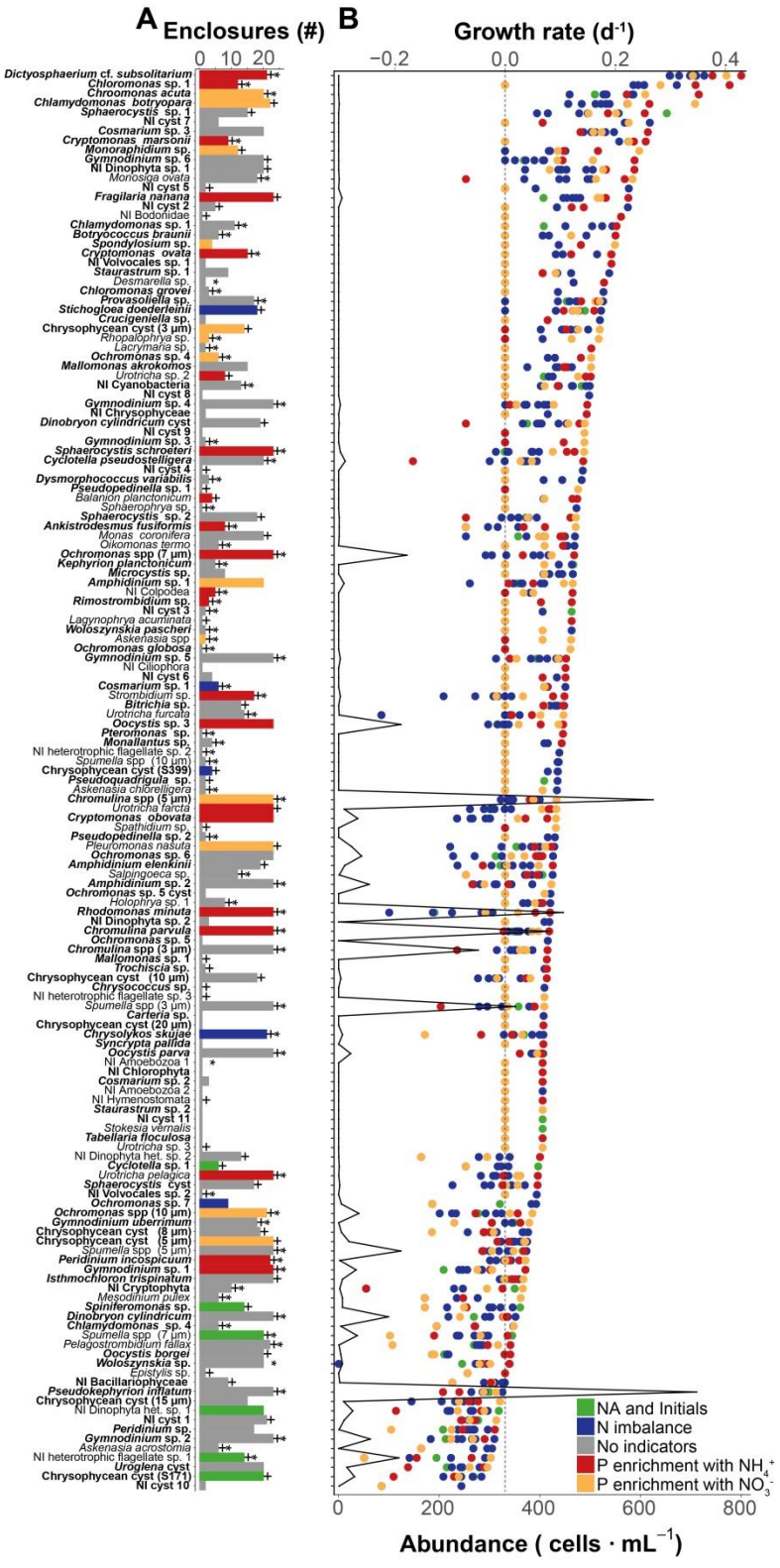

Figure 3.1. Taxa found across the experiment ordered by their maximum growth in any of the treatments. **A**) Number of occurrences in the experimental enclosures and initial samples. Colour bars highlight the indicator value of the taxa to some treatment; + and * indicate the taxa found in the seasonal lake Redon studies of Felip *et al.* (1999a) and Felip *et al.* (1999b) respectively. **B**) Taxon growth rates in each treatment. The abundance of each taxon in the initial samples is shown as a reference (black line).

More than 50% species increased growth at many (if not all) P additions, indicating a general P limitation. This feature was evident when the species were pooled into predominantly autotrophs and heterotrophs guilds (Supp. S3.2, Fig. S3.1). Both categories showed increasing biomass along the P enrichment gradient and no response or decline in the N imbalance gradient. Prokaryotes responded similarly. Therefore, phosphorus addition stimulated growth in all interaction networks, not only autotrophs. Two response patterns were found across the P enrichment gradient concerning the major taxonomic groups (Supp. S3.2, Fig. S3.2, S3.3). The largest biovolume increase of chrysophytes and dinoflagellates occurred from low to medium P enrichment; conversely, cryptophytes, diatoms and chlorophytes and heterotrophs showed a sustained increase across the P enrichment gradient. Two response patterns of the autotroph taxonomic groups were also detected in the N imbalance experiment (Fig. S3.2). The biomass of chrysophytes, dinoflagellates and cryptophytes declined with increasing N imbalance; whereas diatoms, chlorophytes and heterotrophs showed similar biomass values along the gradient. Beyond these general gross patterns, many species showed idiosyncratic responses as the MCVRE –MRT revealed.

The classification of the species response using MCVRE -MRT resulted in five significant progressive splits that accounted for 55% of the total taxon variation (Fig. 3.2). The first split was defined by the degree of P enrichment, splitting the enclosures of the two highest P additions from the rest of the treatments. The second split isolated the treatments with no additions and the initial water column samples of reference from the rest. Therefore, at this level, there were three groups with increasing P availability. Interestingly, the third split was not further related to P levels, but the N source present in the two highest P enrichments (ammonium or nitrate). The fourth split separated those treatments with higher ammonium addition (NH++_P and NH+_P) and also, in another branch, the one with the highest nitrate addition (NO++_P) from the group of the N imbalance treatments. The first and second split were the two with higher increase in explained variation. The fifth split considered the high nitrate treatments, with little further variation explained. The indicator taxa of the response to treatments were determined using IndVal and permutation tests. Forty-five taxa were indicators of clusters at some level of the tree (Fig. 3.2, Supp. S3.3).

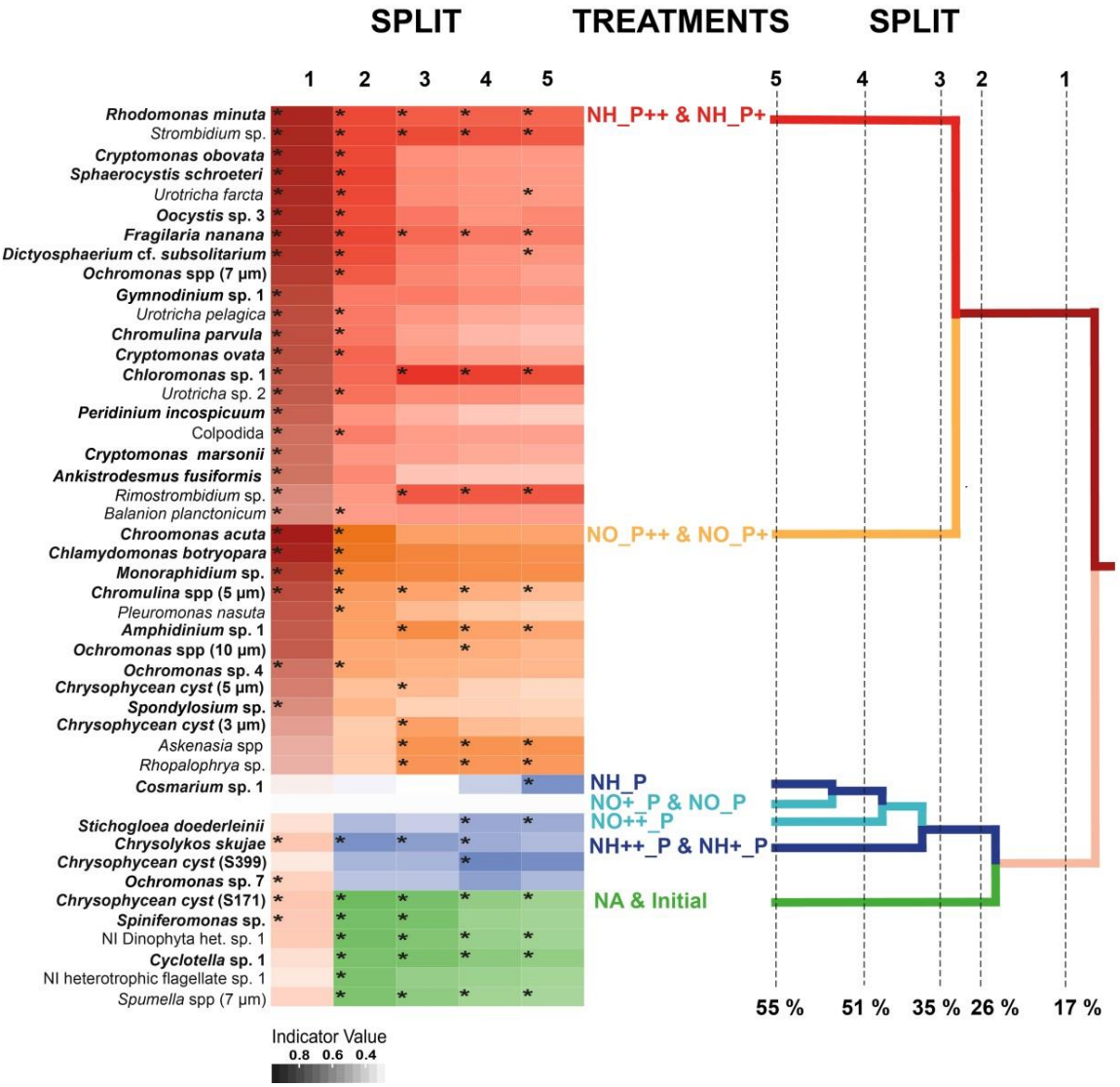

Figure 3.2. Indicator taxa of the community response to nutrient additions as assessed by a multivariate regression tree (MRT). Autotrophic taxa are highlighted in bold characters. Asterisks indicate the splits in which a taxon was significantly indicator of a cluster. Colour intensity indicates the degree of indicator value. The accumulated amount of variance explained at each split is indicated.

3.4.2 Stabilising niche differentiation

Most of the taxa showed positive growth in at least some of the enclosures. Only 13 taxa (8.6%) declined in all treatments. The maximum growth rates were achieved by the initial species with lower biovolume, irrespectively if they were or not indicator taxa at some treatment level (Fig. 3.1). This was not a spurious result due to the use of "estimated" initial density values for the rare taxa in the growth rate calculations, since excluding these initially unseen taxa, the patterns were maintained. Among the cluster indicator taxa, there were both abundant and rare taxa at the beginning of the

experiment. Therefore, the response to the different additions was not conditioned by the initial community structure.

The generalised lower growth of the initially more abundant taxa - even in the best nutrient conditions for the species (Fig. 3.3) – suggest strong influences of stabilising niche differences and relative fitness differences. Among autotrophic organisms, both indicator taxa and neutral taxa showed non-linear maximum growth rate decay with increasing initial population biomass (Fig. 3.3). When a population tended to zero biomass, doubling times tended to 2-4 days, whereas when biovolume was above 1000 μm^3 mL^{-1} the doubling times quickly raised to several weeks or months (Fig. 3.3).

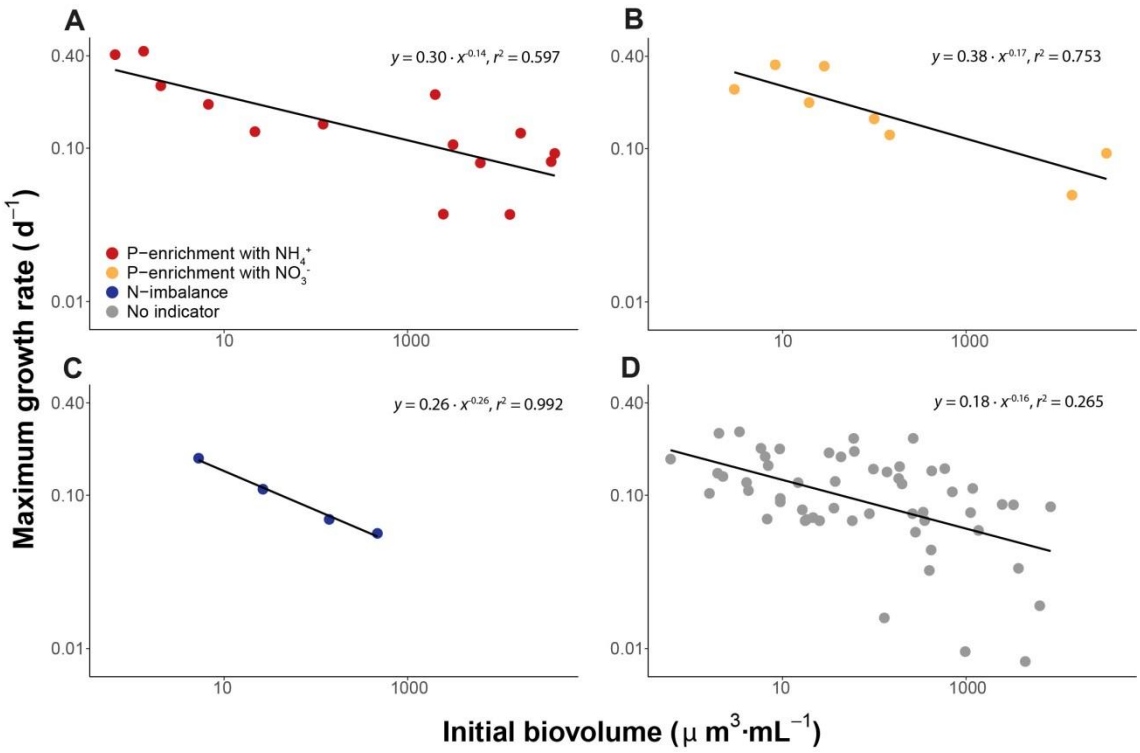

Figure 3.3. Relationship between the initial biomass and the maximum growth rate achieved by each autotrophic taxon. Panels differentiate taxa indicator of the clusters in the third split of the MRT (Fig. 3.3) and no indicators (neutral) taxa.

There was a negative relationship between the proxies of relative fitness (i.e., maximum growth rate) and niche differentiation (i.e., negative average growth rate correlation) for autotrophic taxa (Fig. 3.4). P enrichment indicator species tended to show higher relative fitness and lower niche differentiation than indicators of N imbalance.

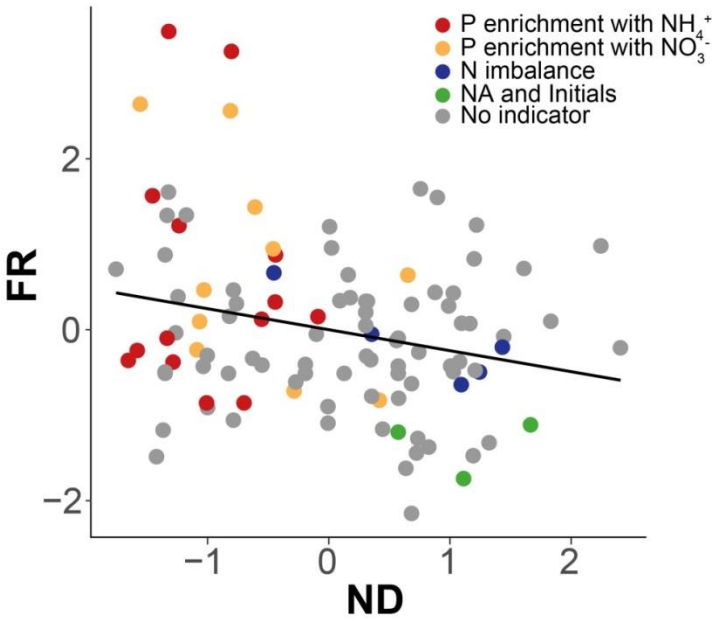

Figure 3.4. Relationship between respective proxies of relative fitness (RF) and niche differentiation (ND) for autotrophic organisms in the experiment. RF was assumed proportional to the maximum growth rate in any of the treatments and ND to the complementary of the average correlation of the growth rate of the species with the rest. Both variables were standardised for comparison. The line indicates a linear fit (R=-0.246, p =0.01).

3.4.3 Chrysophyte encystment

Dinobryon cylindricum was the only abundant chrysophyte species for which we could relate cyst and vegetative forms. The degree of encystment increased in consonance to the enrichment level in both P enrichment and N imbalance treatments (Fig. 3.5 A, B). The highest cyst/vegetative cells ratios were found in the highest P enrichment and N imbalance treatments regardless of the N source. There was substantial variability in the number of chrysophyte cysts in the water column of the enclosures, but on average the density was significantly lower in the ammonium P enrichments (Fig. 3.5 C, D). The MRT analysis indicated that the chrysophyte cysts of 5 µm and 3 µm, appeared related to NO_P+ and NO_P++ and chrysophyte cyst S399 to NH+_P and NH++_P (Fig. 3.3).

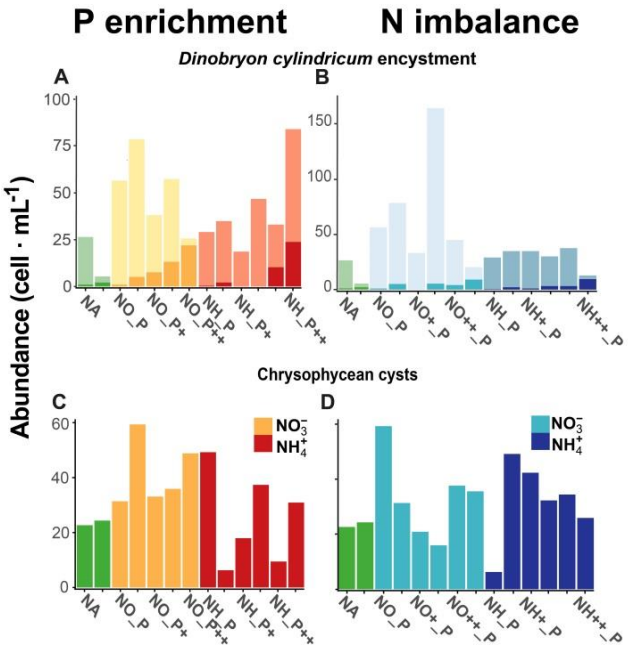

Figure 3.5. Chrysophyte encystment. Abundance of *Dinobryon cylindricum* cells (light colour) and proportion showing encystment (dark colour) in the P enrichment (**A**) and N imbalance (**B**) treatments. Abundance of total chrysophyte cysts in the P enrichment (**C**) and N imbalance (**D**) treatments.

3.5 DISCUSSION

The number and composition of the total taxa recovered from the apparently species-poor epilimnetic waters during the experiment were close to the total taxa found in the two years study of the ice-free period in Lake Redon (Felip *et al.* 1999a) and another study during the ice-covered period (Felip *et al.* 1999b). Most of the species that were not observed in the initial samples were species that were found deeper in the water column during August (i.e., at the hypolimnetic deep chlorophyll maximum) or later in the season in the study of the 1996-97 ice-free periods. We can infer that those species are also periodically recovered when episodic nutrient fluctuations are naturally occurring along the year. In Lake Redon, these episodes are related to water mixing interaction with the sediments during spring and autumn (Ventura *et al.* 2000); nitrification under the ice and ice cover thawing (Catalan 1992a); inflow of snow melting water from the catchment (Catalan 1989) and atmospheric events of dust deposition (Camarero & Catalan 2012). This kind of seasonal nutrient fluctuations are common to many cold dimictic lakes throughout the northern hemisphere (Catalan *et al.* 2002), and episodic fluctuations in nutrients are common in all lakes (Wilhelm & Adrian 2008; Ding *et al.* 2017) and marine systems (Calil *et al.* 2011; Romero *et al.* 2013). Unexpectedly, we recovered some rare litostomate ciliates (*Lacrymaria* and *Lagynophrya*) that previously were observed during winter in the slush layers of the snow and ice cover of the lake (Felip *et al.* 1999b). Because of the low density and only occurrence in some treatment samples, these species did not pass the IndVal randomisation test. Nevertheless, the nutrient conditions in the slush layers, where they were previously found, showed high ammonium and P concentrations, similarly to the enclosure conditions where they appeared.

Nutrient additions have been traditionally used to reveal fitness differences among co-occurring autotrophic species. The increase in the supply rate of a limiting resource should lead to an increase in the abundance of the species that are most limited by the resource (Tilman *et al.* 1982). In our case, the protist community was clearly limited by phosphorus and, therefore, the response along the P addition gradient revealed the relative fitness differences. Most species showed positive growths with the additions. In some experiments with plants, N addition is accompanied by diversity loss, suggesting that the addition is also affecting the stabilising niche differences (Clark & Tilman 2008). This was not our case because the observed species diversity was similar or higher in the additions than in the initial water samples. Notably, there was phylogenetic clustering of the main groups across the P axis (i.e., chrysophytes and dinoflagellates at low P and cryptophytes, diatoms and chlorophytes at high). Contemporary coexistence theory interprets this kind of patterns as reflecting relative fitness differences (competitive ability) or environmental filters (Mayfield & Levine 2010). The two mechanisms are not easily distinguishable and, in fact, are not mutually exclusive. Environmental filtering will drive phylogenetic clustering when relevant

traits are sufficiently conserved and closely related taxa share similar environmental tolerance. The increase of *D. cylindricum* encystment at high P and N imbalance is an indication of limited environment tolerance, despite that fundamental resources such as N or P were highly available. Nevertheless, the predominance of chrysophytes over cryptophytes and diatoms at low P are more likely related to the competitive ability. In both cases, the clustering of the main taxonomic groups across the P gradient will indicate niche conservatism, concerning P availability, of most of the taxa in the group. This feature is well-known in freshwater phytoplankton (Reynolds 2006), which has been used to develop trophic state indicators (Phillips *et al.* 2013). The relative fitness differences do not promote coexistence but competitive exclusion (Tilman *et al.* 1982). On the contrary, the sensitivity of chrysophytes and dinoflagellates to the N imbalance will be a stabilising niche difference that may favour the coexistence between the major groups in an environment of fluctuating stoichiometry of the inorganic nutrient resources.

In addition to the N imbalance, the N source that accompanied the P availability can be particularly relevant as a stabilising niche difference. In our experiment, once the P was relatively available, the main factor of community differentiation was the N source form, either nitrate or ammonium. The maximum growth rates achieved by the species and the total biomass growth were similar for the two N sources and dictated by the amount of P, but the species involved were different. Resource partition can be one of the main mechanisms of coexistence and is non-dependent on environmental fluctuations (Chesson 2000). In Lake Redon, and similarly, in other P limited lakes, nitrate is usually more abundant than ammonium as the N source. Nonetheless, large increments of phosphorus are usually associated with ammonium if the P sources are sediments (Ventura *et al.* 2000) or atmospheric deposition (Camarero & Catalan 1996). Nowadays, it has been realised that the N source preference for phytoplankton is a complex issue that can be species-specific (Glibert *et al.* 2016). The MRT results showed that species do segregate according to the N source with the same P availability. The MRT clusters indicator of high P with ammonium as N source showed a few more indicator species than those with nitrate. Even so, all main taxonomic groups of autotrophic protist showed indicator species in both N source clusters. In a study of the stomatocysts distribution across a large number of Pyrenean lakes (Pla *et al.* 2003), nitrate was a factor explaining a significant percentage of the chrysophyte cyst composition. This overdispersion of chrysophycean species across the ammonium and nitrate availability also indicates a stabilising niche difference.

The maximum per capita population growth rates that autotrophic species achieved in the addition experiments showed a negative relationship with their biovolume in the initial lake conditions. Also, these rates showed a negative correlation with a proxy for niche differentiation (i.e., the average correlation of the species growth with the rates of

other species). Both patterns are indicators of stabilising niche differences. The negative slope of the relationship between per capita growth rate and frequency (i.e., initial abundance in the experiment) can be used as a quantification of the stabilisation (Adler *et al.* 2007). The slope of the relationship was similar among groups of indicator species of high P enrichments - either ammonium or nitrate-, nitrogen imbalance and without specific response clusters. Therefore, there are no major differences in using one or another N source for remaining in the community at long-term. Each of these clusters could be considered a guild that uses N and P nutrients similarly. Belonging to different N guilds will be a stabilising mechanism for P competition. Species less competitive in the P gradient should show higher niche differentiation, as indicated by the proxies used. The buffered population growth found within each of the guilds indicates that there were also other stabilising niche differences acting. Such mechanisms may include partition respect to other nutrients, storage effects and prey-specific predation.

Storage effects can be the dominant coexistence mechanisms in fluctuating environments (Angert *et al.* 2009). The storage effect combines species-specific response to the environment and population-dynamics buffering by the presence of a persistent stage during low resource periods. The persistent stage is a way to sustain the population by having high survival over periods of low resources and fuel fast population growth when favourable (Li & Chesson 2016). It may take many forms. The simplest persistent stage is a remaining population with low competition because of the very low relative densities compared to other species and the specific demographic potential of the species. On the other hand, this remaining population has to be sufficiently high in absolute numbers of individuals to be far from stochastic extinction. In the lake protist realm, where the populations oscillate many orders of magnitude, extremely low relative populations still include many millions of individuals. Low frequency also constitutes a refuge for preys sharing predators (Abrams & Matsuda 1996); and thus survival increases. Hence, storage effects can be easily achievable by protists populations without specific adaptations. Nevertheless, some protist groups do show specialised resting stages.

Our experiment focused on recovering species from the epilimnetic waters using small enclosures. However, the true framework for coexistence is the whole lake. The storage effect emphasises a persistent stage in the life cycle (Chesson 1983). When environmental conditions are poor, the reproductive effort is reduced, and energy investment goes to survivorship. This persistent stage can take the form of resting stages that sustain the population over periods of poor recruitment and fuels the population growth when favourable (Li & Chesson 2016). Many planktonic organisms produce resting stages (e.g., Dinophyta, Synurophyceae, Phaeotamniaceae Bacillariophyceae, among others) (Ellegaard & Ribeiro 2018). In our experiment, the

chrysophyte cysts were particularly conspicuous, and the case of *D. cylindricum* follows the theoretical predictions: the population growth declined, and the encystment rate increased at high P and N additions. Our results show that the proportion of encystment increase when the intra-specific competition is higher. In this way, cyst formation is a life-history trait that enhances buffered population growths and thus coexistence. *D. cylindricum* is one of the few species for which the conditions of cyst development have been studied experimentally. Sandgren (1983) already stated that cyst could be formed due to population density and later indicated a threshold that triggered cyst development (~200 cell mL^{-1}) from which the proportion of encysting cells increased (Sandgren 1988). In our case, the threshold and high encystment occur at lower cell densities (Fig. 3.5). Unfortunately, the connection between chrysophyte vegetative forms and their cysts have not been established except for a few species. Chrysophytes biomass did not show a significant difference to P addition when ammonium or nitrate was the N source, although the species mostly reacting were not the same for the respective sources. In contrast, there were differences in the encystment rate, which were higher with nitrate. Consequently, the stabilizing niche differences include not only resource partition but also differentiation in the way the persistent stages are regulated.

Low overlap in predators acts as a stabilising niche mechanism. Predation does not work upon the results of the competition for resources but at the same level as resources (Chesson & Kuang 2008). Species limited solely by predation, rather than resources, can coexist if they differ sufficiently in the extent to which different predators limit them relative to how much they differ in competitive ability (Kuang & Chesson 2010). In our results, we found that the heterotrophic protist biomass was highly coupled to the autotrophic response to the treatments. The coupling could be partially due to direct interaction and partially mediated by prokaryotic growth, which also changed coherently with autotrophs. The parallel autotrophs-heterotrophs biomass response could suggest low differential predation. However, the IndVal tests revealed many indicator heterotrophic taxa in the MRT clustering. Selective predation among protists or upon prokaryotes is another source of niche differentiation co-occurring with nutrient partition. Noteworthy is the fact that heterotrophic protists clustered as indicator species together with the autotrophic organisms. Actually, the response in biomass to P additions was similar from prokaryotes to ciliates; the enhanced growth of primary producers propagated to the whole microbial food web. Therefore, even we could consider guilds of a similar lifeform as competitors; the fact is that they are not independent of other guilds and their interactions act as stabilising niche difference. For instance, among bacterivores, there are heterotrophic and autotrophic (mixotrophic) organisms and, even within the latter, selective predation has been described (Ballen-Segura *et al.* 2017).

The coexistence in planktonic protist community cannot be seen exclusively through pairwise competition mechanisms within closed guilds; rather, it is closer to a complex structure of diverse competitive networks that partially overlap (Levine *et al.* 2017). The planktonic protist community is a network of weak and strong interactions. Strong interactions related to the use of limiting nutrients or shared preys may define relative fitness differences, whereas the myriad of weak ones (e.g., alternative N source, stoichiometric requirements, prey selection) may contribute to the stabilising niche differences. Merging network and coexistence theory seems to be the way forward (Saavedra *et al.* 2017). Extremely close species may have relatively small differences, but they also will require small stabilising niche differences to maintain coexistence (Levine *et al.* 2017). In mountain lakes, the coexistence of many chrysophycean species with similar P and N source requirements does not need to be seen as a paradox. Small differences in sensitivity to N imbalance, cyst formation regulation, specific predators, prey selection as mixotrophs and to physical constraints quickly may balance the relative fitness differences concerning P uptake ability. The fluctuations of the environment and the protist populations in mountain lakes are huge; these fluctuations offer an opportunity for species coexistence rather than limiting them because of the phosphorus or nitrogen scarcity. More productive or larger aquatic systems with alternating or co-limiting nutrients may sustain even richer protists communities because average per capita growth rate differences between species over long periods would be harder to achieve. Changes in the limiting resource may occur more often and, on the other hand, environmental and competitive covariation should be lower than in oligotrophic sites, enhancing stabilising niche differences.

3.6 SUPPORTING INFORMATION

Supplementary material S3.1: Sample collection and protist analysis

The enclosures were deployed and treated on 5-6 August 2013 and recovered 25 days later. Integrated water samples (~ 5 L) were collected deploying 20 m-long plastic tubes inside the enclosures and pumping the water in them (Giménez-Grau *et al.* submitted-a). The water was immediately filtered through a 250 μm-pore size mesh to collect large zooplankton. As expected, the total number of crustacean individuals in each enclosure was low (5 individuals on average): 50% were *Cyclops abyssorum* adults, 40% other stages of these species including nauplii, 3% *Diaptomus cyaneus* and 7% *Daphnia pulicaria*. Indeed, none of the measured variables in the enclosures showed any significant relationship with the crustaceans present. Several water subsamples were allocated for chemical and biological analyses. Specifically, 200 ml subsamples were preserved with 0.5% (vol/vol) alkaline Lugol's solution for protist community analysis (Sournia 1978), and 50 ml subsamples were preserved with 2% (vol/vol) formaldehyde for prokaryote evaluation (Porter & Feig 1980). The sediment traps were collected by gently uplifting and emptying the bag part of the enclosures without disturbing the sediment traps. After removing the plastic bag, the sediment trap content was decanted into a plastic bottle and kept until filtration in the laboratory. An integrated water sample of the epilimnion and upper metalimnion, from 0-20 m depth, was collected on 6 August 2013 to assess the initial conditions of the experiment.

The protist abundance was evaluated microscopically according to the Utermölh technique (Sournia 1978). The protist biovolume was estimated adjusting appropriate geometrical forms to the species shape (Hillebrand *et al.* 1999). For chrysophyte cyst analyses, the filters from the sediment traps were treated with 33% hydrogen peroxide and mounted in Naphrax (Battarbee *et al.* 2001). Cysts were identified according to Pla (2001) and concentrations estimated by the addition of a known number of microspheres (Battarbee & Kneen 1982). The abundance of prokaryotes was determined by epifluorescence microscopy, using DAPI staining on 0.2-μm pore size filters following the technique described in Porter and Feig (1980). For each subsample, two replicate filters were processed.

Supplementary material S3.2: Functional and taxonomic group response

Autotrophic and heterotrophic organisms showed similar biomass response to the treatments by the two groups (Fig. S3.1). The abundance of prokaryotes also responded to the treatments with patterns similar to eukaryotes (Fig. S3.1 C, F).

Autotrophic and heterotrophic biovolume increased proportionally to P availability. About four-fold higher biovolume was achieved in the highest P enrichments than in the lowest ones. The proportion between the two groups across P enrichments showed marked stability, with autotrophic organisms being 80-90 % of the total biovolume (Fig. S3.1 A and S3.1 B, bar plots). Regarding the different dissolved inorganic nitrogen source, we did not find differences between P enrichment treatments neither for autotrophs, heterotrophs, nor prokaryotes.

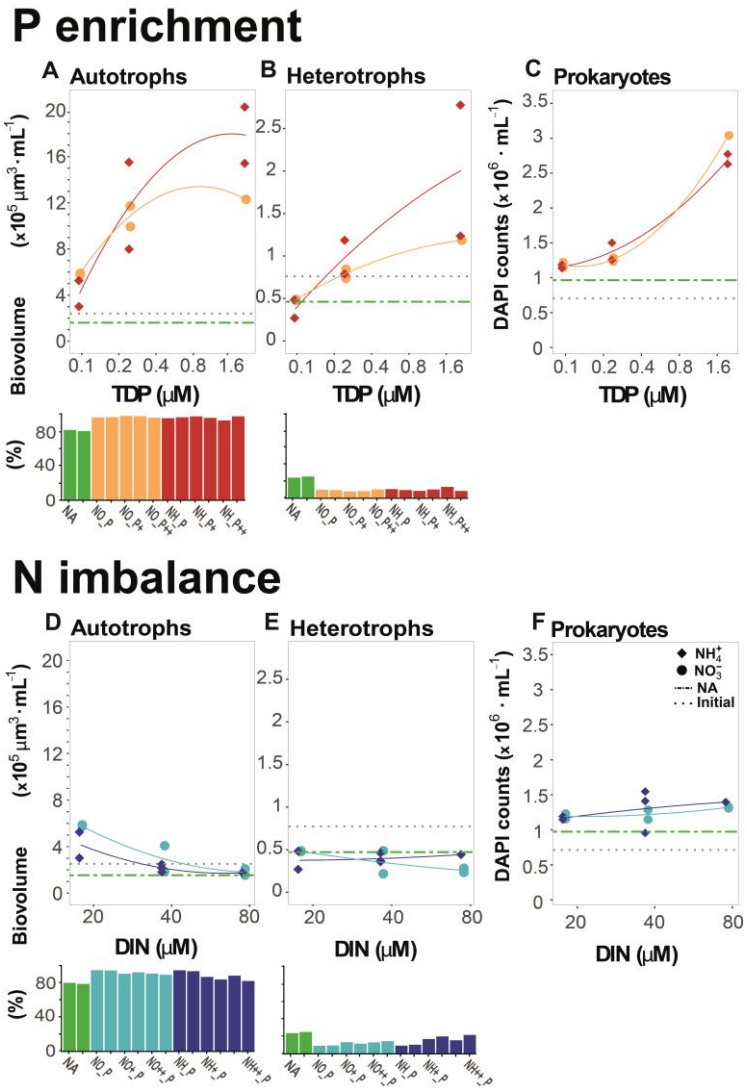

Figure S3.1. Microbial response to P enrichment and N imbalance grouped by autotrophic protist (A, D), heterotrophic protist (B, E) and prokaryote (C, F) organisms. Mean values of the lake initial conditions and the enclosures without nutrient additions are included as references. Bottom bar diagrams indicate the proportion between the two trophic groups in each of the treatments. Red and dark blue colours refer to treatments with ammonium as the nitrogen source, orange and light blue to treatments with nitrate and green to enclosures with no addition (NA). Tendency lines were fitted using LOESS.

For the N imbalance treatments, autotrophs declined proportionally to the DIN addition, while heterotrophs did not show differences between treatments (Fig. S3.1 D and S3.1 E). Consequently, the biomass proportion of autotrophs slightly decreased at higher N imbalance. As in the P enrichments, no differences were found between DIN forms (NH_4^+ and NO_3^-).

The general common response is an indication of the close functional links between the whole planktonic microbial community or, at least, between the elements contributing the most to the community biomass. Autotrophs' growth provides fresh carbon for heterotrophic prokaryotes, which fuel the many heterotrophic protists that are bacterivores. Also, a few protists were observed to predate on other protists during counting (e.g., heterotrophic dinoflagellates on diatoms) and many other cases are

possible. There were many mixotrophic species – autotrophs with phagotrophic ingestion of prokaryotes – within chrysophytes, dinoflagellates and cryptophytes main groups. Therefore, photoautotrophic and heterotrophic protist cannot be considered independent guilds, and phosphorus limitation extends to the whole protist community.

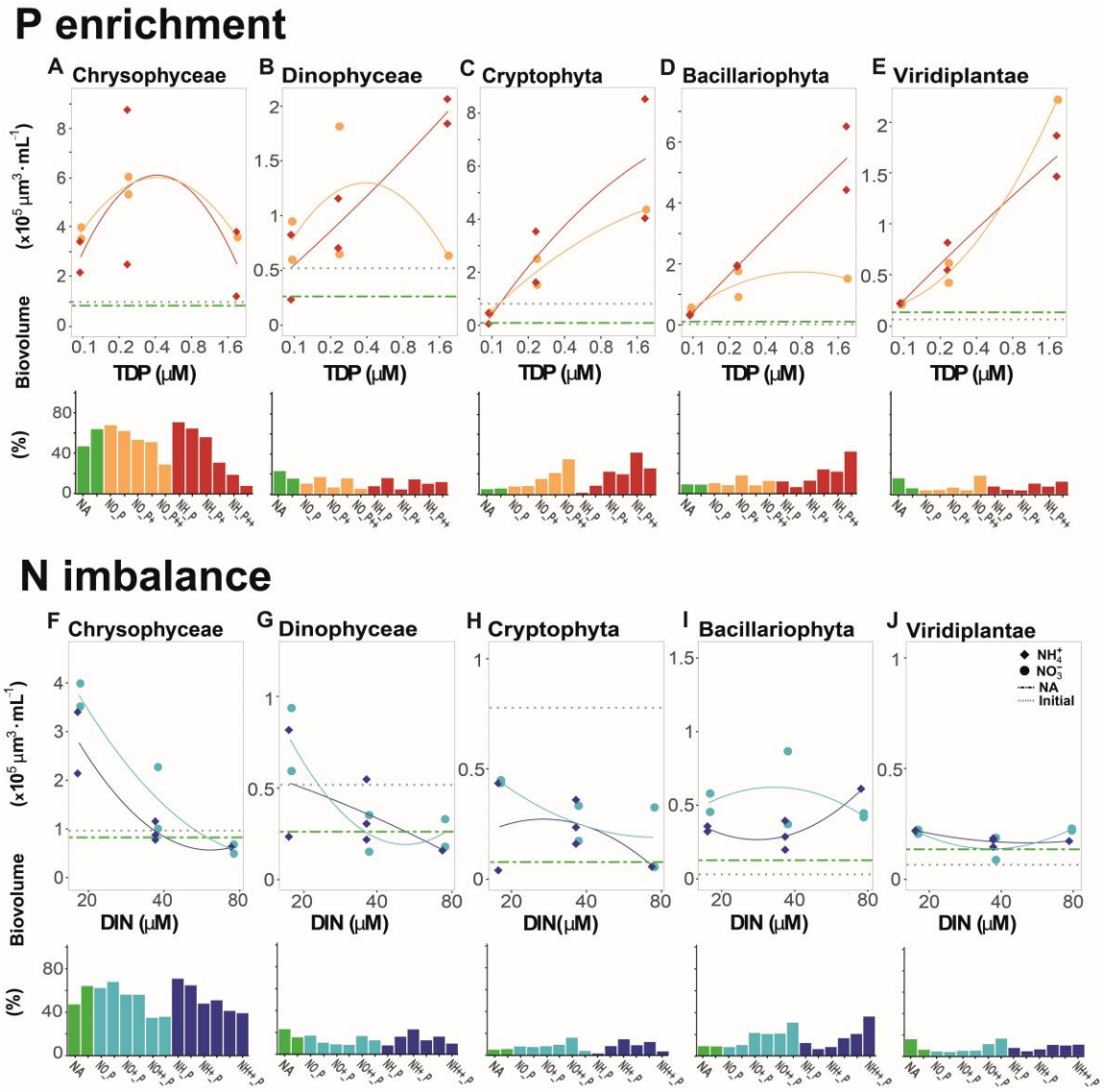

Figure S3.2. Autotrophic protist response to P enrichment and N imbalance grouped by the main taxonomic groups (chrysophytes, dinoflagellates, cryptophytes, diatoms and chlorophytes). Mean values of the lake initial conditions and the enclosures without nutrient additions are included as references. Bottom bar diagrams indicate the proportion between the two trophic groups in each of the treatments. Red and dark blue colours refer to treatments with ammonium as the nitrogen source, orange and light blue to treatments with nitrate and green to enclosures with no addition (NA). Tendency lines were fitted using LOESS.

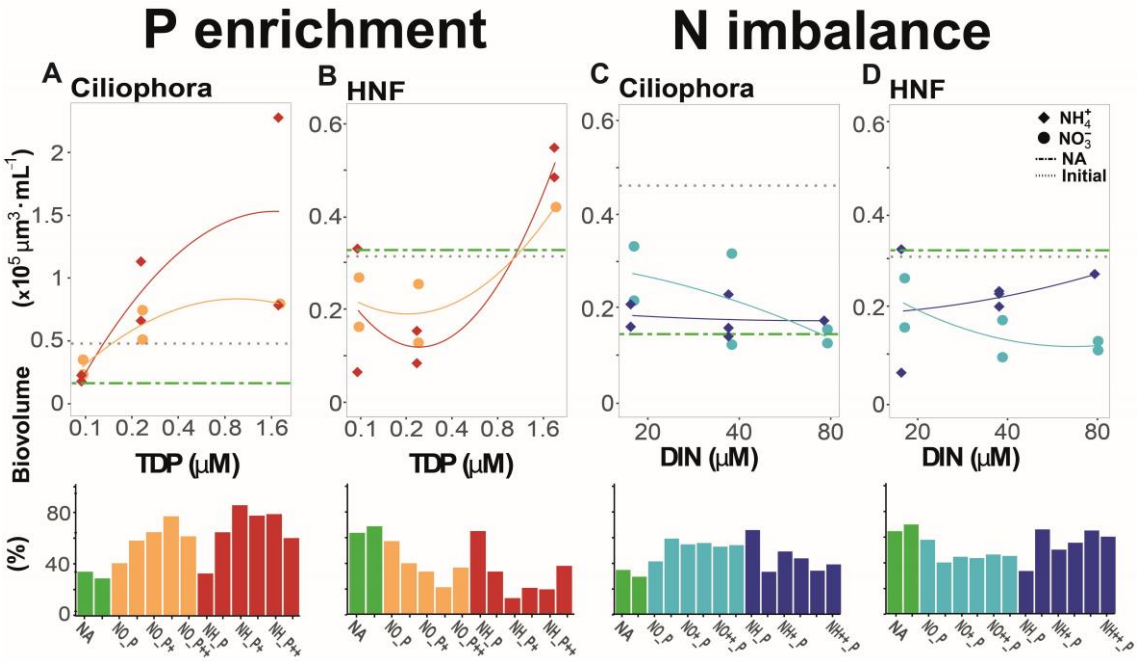

Figure S3.3. Biomass (biovolume) protist response to P enrichment and N imbalance grouped by the main heterotrophic groups (ciliates and heterotrophic flagellates). Mean values of the lake initial conditions and the enclosures without nutrient additions are included as references. Bottom bar diagrams indicate the proportion between the two trophic groups in each of the treatments. Red and dark blue colours refer to treatments with ammonium as the nitrogen source, orange and light blue to treatments with nitrate and green to enclosures with no addition (NA). Tendency lines were fitted using LOESS.

The main taxonomic (phylogenetic) groups largely also correspond to the main functional lifeforms in highly oligotrophic lakes (Reynolds *et al.* 2002). However, there are some predominantly autotrophic groups (i.e., chrysophytes, dinoflagellates and cryptophytes) that also include some species that are exclusively heterotrophic. We considered all these latter exceptions in a single differentiated group, heterotrophic nanoflagellates (HNF).

Two response patterns were found among the autotrophic groups across the P enrichment gradient (Fig. S3.2). The largest biovolume increase of chrysophytes and dinoflagellates occurred from low to medium P enrichment presenting a saturation tendency at high P enrichment (Fig. S3.2 A, B); except when the N source was NH_4^+, in which case dinoflagellates continued increasing at high P enrichment. Conversely, cryptophytes, diatoms and chlorophytes showed a sustained increase across the P enrichment gradient (Fig. S3.2 C, D, E). Chrysophytes were the most abundant group at low P enrichment (~ 60 % of the autotrophic biovolume) and declined until 10 % at the highest P enrichment (Fig. S3.2). The patterns were similar for both N sources. Cryptophytes and diatoms presented an opposed pattern to chrysophytes, increasing the relative biovolume from <10 % to >40 % as P enrichment increased. In the case of

diatoms, only when NH_4^+ was the main N source. Dinoflagellates and chlorophytes showed little relative changes, always below 20 % of the autotrophic biovolume.

Two response patterns of the autotroph taxonomic groups were also detected in the N imbalance experiment (Fig. S3.2). The biomass of chrysophytes, dinoflagellates and cryptophytes declined with increasing N imbalance; whereas diatoms and chlorophytes showed similar biomass values along the gradient. The most pronounced decline was shown by chrysophyceae, declining from 60 % of the relative biovolume to 30 %. Diatoms were the unique group that showed a relative increase from 9 % to 35 %, although they did not experience biomass increase.

Among heterotrophic species, we only distinguished between ciliates and the HNF taxonomically miscellaneous group. Both groups increased in the P enrichment and were relatively stable in the N imbalance (Fig. S3.3). The increase of HNF only occurred at the highest P additions. Although in the enclosures with no additions (NA), the HNF biomass was higher than ciliate biomass, in all P enrichment treatments, the relative biovolume of ciliates was higher (up to 80 %) than the HNF biovolume. In all N imbalance treatments, both groups showed relative biomass around 50 %. No differences concerning the N sources were found.

Supplementary material S3.3: Indicator species

Many of the species (21) were indicators of clusters concerning with high P (P+ and P++) and ammonium as N sources; thirteen of high P with nitrate; five of N imbalance and six more of the enclosures without addition or the initial conditions.

The first two initial splits showed more indicator taxa (30 and 28, respectively) than each of the following splits, which stabilised in 17. All the clusters showed autotrophic and heterotrophic organisms as characteristic taxa. Indicator taxa of the main taxonomic groups appeared across all the main clusters, although some of the groups were particularly represented in some of them. In the high P with ammonium treatments (NH_P++ and NH_P+ treatments), most of the indicator species belonged to the autotrophic groups chlorophytes (*S. schroeteri*, *Oocystis* sp.3, *D. subsolitarium*, *Chloromonas* sp.1, *A. fusiformis*), cryptophytes (*R. minuta*, *C. obovata, C. ovata, C. marsonii*) and diatoms (*Fragilaria nanana*), and the heterotrophic ciliates (*Strombidium* sp., *U. farcta*, *U. pelagica*, *Urotrichia* sp.2, Colpodida, *Rimostrombidium* sp., *B. planctonicum*). Some chrysophytes (*Chromulina* spp (5 μm), *Ochromonas* spp (7 μm)) and dinoflagellates (*Gymnodinium* sp.1, *P. incospicuum*) were also indicators. In contrast, treatments of high P with nitrate as N supply (NO_P++ and NO_P+) had chrysophytes as the most common indicators (*Chromulina* spp (7 μm), *Ochromonas* sp.4, chrysophycean cysts of 5 μm and 3 μm, *Ochromonas* spp (10 μm)). Other main groups

were also represented including taxa from chlorophytes (*C. botryopara*, *Monoraphidium* sp., *Spondylosium* sp.), dinoflagellates (*Amphidinium* sp. 1), cryptophytes (*C. acuta*), ciliates (*Askenasia* spp, *Rophalophrya* sp.) and HNF (*P. nasuta*). There was much fewer species indicator of the N imbalance. Remarkably, when the imbalance was due to ammonium (NH++_P and NH+_P), there were indicator chrysophytes taxa (*C.skujae*, chrysophyte cyst S399, *Ochromonas* sp. 7). The highest nitrate enrichment (NO++_P) was characterised by *Stichogloea doederleinii*, a non-flagellated species, which was placed within chrysophytes until recently, and that has been moved to Phaeothamniophyceae. Most of the indicator taxa in the NA and initial samples were HNF.

Regarding the major taxonomic groups considered, 83 % of the cryptophytes taxa were indicators, 19 % of the dinoflagellates, 40 % of the diatoms, 38 % of the chrysophytes, 28 % of the chlorophytes, 38 % of the ciliates and the 22 % of the HNF. It is also worth to note that some taxa that were not selected as indicator due to their low occurrence (one sample or two at low relative abundance) were highly idiosyncratic and unexpected, particularly, the litostomate ciliates (*Lacrymaria* sp. and *Lagynophrya acuminata*) and the chlorophytes *Pteromonas* sp., which appeared in the NH_P++ and NH_P+ treatments.

CHAPTER 4
Diversity in the response of bacterioplankton to the episodic nutrient enrichment in P-limited oligotrophic conditions

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4.1 ABSTRACT

Cold, well-oxygenated oligotrophic lakes show a rich bacterioplankton community. Seasonal fluctuations in environmental conditions and, particularly, nutrient enrichments may fuel this diversity by direct influence or mediated by the phytoplankton growth and increased availability of labile organic compounds. The ENEX experiment was planned to evaluate the effects of phosphorus (P) and nitrogen (N) enrichments on the planktonic community of a P-limited ultraoligotrophic lake. Here, we report the changes in the bacterioplankton community during the experiment, assessed by 16S rRNA gene amplicon sequences, and compare the results with the bacterioplankton seasonal changes occurring in the lake. In the experiment, bacterioplankton abundance followed the general phytoplankton response, but there were characteristic responses at low taxonomic rank to nutrient treatments. Most groups — and emphatically the Betaproteobacteria families, Comamonadaceae and Oxalobacteriaceae — showed representative OTUs that preferentially responded to either intermediate or high P, nitrate, ammonium or no additions. These OTU idiosyncratic responses can be seen as resource partitioning mechanisms that stabilise the coexistence of otherwise functionally similar competing species. Only the Actinobacteria ACK-M1 showed a clustered response to ammonium enrichments. Some of these ACK-M1 OTUs are abundant and stable throughout the year in the lake. They constitute the background assemblage of the ultraoligotrophic conditions. We may speculate if differential predation by bacterivore protist acts as a stabilizing mechanism of coexistence in this particular group.

4.2 INTRODUCTION

The diluted, cold, well-oxygenated, extremely oligotrophic and carbon poor waters of high mountain lakes show a rich bacterioplankton community throughout the year (Pernthaler *et al.* 1998; Zufiaurre *et al.* submitted-a). Although there are some seasonal fluctuations in the assemblages following the main physical changes (i.e., ice-cover, thawing, spring overturn, summer stratification, autumn mixing and early ice-cover), there is a large number of OTUs that are resistant to the changes and others that recover quickly from unfavourable periods. The main classes and the families within them show a rich number of OTUs. If we assume an ecological coherence of high bacterial ranks (Philippot *et al.* 2010), at least at the family level, then this phylogenetically close OTUs have to be seen as competitors. Long-term coexistence between competitors is only possible if the relative fitness differences between them — which will bring to exclusion and eventually only one winner — are compensated by stabilizing mechanisms that result in niche differentiation in a fluctuating environment (HilleRisLambers *et al.* 2012).

Resource changes accompany physical lake fluctuations. There are external and internal nutrient loadings and changes in phytoplankton growth that modify the availability of labile organic carbon for heterotrophic bacterioplankton (Catalan *et al.* 2002). We may hypothesize that those fluctuations, within the general context of oligotrophy, may favour resource partitioning that stabilizes coexistence between otherwise similar organisms.

The ENEX experiment was planned to investigate the effects of phosphorus (P) and nitrogen (N) episodic enrichments in the planktonic community of a P-limited ultraoligotrophic lake (Giménez-Grau *et al.* submitted-b; Zufiaurre *et al.* submitted-b). In this experiment, the episodic nutrient enrichments were simulated in field enclosures in which additions were applied to obtain variation in the degree of P enrichment, the N imbalance respect the limiting P and the N supply (NH_4^+ or NO_3^-). Here, we investigate how the bacterioplankton assemblages changed with the treatments and compare the changes with those that have been described throughout the annual cycle in the same lake (Zufiaurre *et al.* submitted-a). The aims are (i) to assess if OTUs of the same high taxonomic rank (class, family) cluster around some treatment or, alternatively, spread across some of them and (ii) check if the bacterioplankton responses found in the experiment are coherent with the field observation during the annual cycle.

4.3 MATERIAL AND METHODS

4.3.1 Lake Redon and the ENEX experiment

Lake Redon is a high mountain ultraoligotrophic lake located in the central Pyrenees (42º38'33" N, 0º36'13" E, 2232 m a.s.l.) (Box 1). It has an area of 24 ha and a maximum and mean depth of 73 m and 32 m, respectively, with a water average residence time of ~4 years (Catalan 1988). The lake is dimictic and covered by ice during six-seven months per year. The photic zone (40-50 m) largely exceeds the seasonal thermocline (~15 m depth) during the summer stratification, and the epilimnion shows low chlorophyll levels compared to the upper hypolimnion (Felip & Catalan 2000). There is a large imbalance between P and N availability (Catalan 1992a) due to the high nitrogen deposition in the area (Camarero & Catalan 1996), although increased P atmospheric inputs have alleviated the difference (Camarero & Catalan 2012). Seasonal changes in primary production are mainly driven by internal nutrient loadings during spring and autumn mixing periods (Catalan *et al.* 2002; Pla-Rabes & Catalan 2011). Throughout the year, N:P ratios and the proportion between ammonium and nitrate change, mainly related to snow thawing loads, under ice processes (e.g., nitrification) and sediment exchanges (Catalan 1992a; Ventura *et al.* 2000).

The phytoplankton community is dominated by chrysophytes, with occasional relevance of species belonging to other groups during the mixing periods (diatoms and chlorophytes), summer stratification (dinoflagellates) or in deep layers (cryptophytes). Heterotrophic protists (i.e., ciliates and heterotrophic nanoflagellates (HNF)) can achieve biomass levels larger than autotrophs during some periods (Felip *et al.* 1999a). Recently, the bacterial community has been studied throughout the seasonal cycle (Zufiaurre *et al.* submitted-a). The study included samples collected every four weeks, from January 2013 to January 2014, at 2, 10, 20, 35 and 60 m depths. The analytical procedures were identical to those described below for the ENEX experiment. The data from this study are evaluated again here as a complement to the interpretation of the bacterioplankton results in the ENEX experiment.

The ENEX experiment aimed to test the role of episodic nutrient enrichments in the plankton dynamics; in particular in the coexistence of species (Zufiaurre *et al.* submitted-b) and stoichiometric changes (Giménez-Grau *et al.* submitted-b). Here, we investigate the effects on bacterioplankton. We performed pulse nutrient perturbations of the summer epilimnetic protist community using enclosures of 100 L that consisted of self-filling 20 m-long transparent cylindrical bags closed by a sediment trap, which were vertically deployed, and in which nutrient additions were performed. Construction, deployment, manipulation and performance are detailed in Giménez-Grau *et al.* (submitted-a). Eleven treatments were performed with two replicates; thus, 22 enclosures were deployed. The TP, nitrate and ammonium concentrations selected were within the oligotrophic seasonal and interannual variation found in the lake and snow cover, plus some extreme treatments above this range, providing mesotrophic conditions according to TP and nitrogen imbalance levels of regions highly affected by N atmospheric contamination. Both P and N were added to each treatment to avoid that the addition of only one nutrient could induce the other to become markedly limiting at some point during the experiment. We established a gradient of P additions ("P enrichment") and another of N ("N imbalance") (Table 1.1). Two enclosures with no nutrient addition (NA) were used as a reference for the enclosure effect. Unfortunately, one replicate of the NO_P++ treatment was lost during the field experiment. The enclosures were deployed and treated on 5-6 August 2013 and sampled 25 days later. Integrated water samples (~ 5 L) were collected deploying 20 m-long plastic tubes inside the enclosures and pumping the water in them (Giménez-Grau *et al.* submitted-a). The water was immediately filtered through a 250 μm-pore size mesh to remove large zooplankton, and several water subsamples were allocated for chemical and biological analyses. For the chemical analyses, water samples were filtered through GF/F fibreglass filters for dissolved nutrient and chlorophyll analyses. For bacterioplankton DAPI counts, immediately after sampling, 100 ml were fixed with formalin (final conc. 2 % v/v). For 16S rRNA analyses samples were filtered until filter

saturation using a 47 mm diameter and 0.2 μm pore size polycarbonate filters and preserved with lysis buffer at -80 ºC until DNA extraction.

4.3.2 Chemical analyses

Concerning the ENEX specific data reported here, dissolved organic carbon (DOC) was determined by catalytic combustion and infrared spectrometric detection of the CO_2 produced (TOC5000 Shimadzu analyser) and Chla was extracted in 5 ml 90% acetone with a probe sonicator (Sonopuls GM70 Delft, The Netherlands) (50W, 2 min), and the extracts were subsequently centrifuged (4 min at 3000 rpm, 4 ºC) and filtered through a Whatman Anodisc 25 (0.1 μm). Chla was analysed by ultra-performance liquid chromatography (UPLC, Acquity, Waters, Milford, MA, U.S.A.) as reported in Buchaca et al. (2005). Details on other chemical analyses are provided in Giménez-Grau *et al.* (submitted-b) for the ENEX experiment and Zufiaurre *et al.* (submitted-a) for the seasonal study, which were fundamentally the same.

4.3.3 Bacterioplankton analyses

Prokaryotic abundance was calculated by epifluorescence microscopy (Zeiss Axio Imager, 1000x and a UV-excitation / long-pass emission filter set) using DAPI staining on 0.2-μm pore size polycarbonate filters (Porter & Feig 1980). For each sample, two replicate filters were processed. In each filter, a minimum of 2000 bacterial cells was counted in at least 10 random fields.

The DNA extraction was performed using the Fast DNA SPIN kit (MP Biomedicals, Santa Ana, CA, USA) and quantified using Qbit fluorometer (Thermo Fisher Scientific Inc.). Quantitative real-time PCR (qPCR) was used to quantify 16S rRNA gene, in combination with the primers 341F and 534R (López-Gutiérrez *et al.* 2004) and iQ SYBR Green PCR Master Mix (Taula S4.1). Replicates were conducted on different plates, and the average concentration per each sample was calculated.

Diversity and structure of the bacterial community were determined by the sequencing of the V3-V4 hypervariable region of the 16S rRNA gene. Amplicon libraries for each sample were generated using a two-step Polymerase Chain Reaction (PCR) protocol (Berry *et al.* 2011). The first step consisted in four replicates per sample due to the low yield of the samples, comprising each replicate 15 μL reaction blending 0.1 ng of the sample with 2X Phusion PCR Master Mix, 0.25 μM of each 16S rRNA universal primers Pro805R and Pro341F (Takahashi *et al.* 2014) and 0.5 $\mu g \cdot \mu L^{-1}$ BSA. Thermal cycling was performed for 25 cycles using the Applied Biosystems 2720 thermal cycler (Taula S4.1). Afterwards, replicates were pooled together and purified using the Agencourt

AMPure kit. The second step consisted in 5 replicates, mixing 5 μL of the purified product with 0.2 μM of each Nextera adapter sequences and similar reagent concentrations as in the first step, consisting in 30 μL of reaction. The final products were pooled together and purified following AgencourtAMPure XP method and quantified using Qbit fluorometer (Thermo Fisher Scientific Inc.). Goodness of the product was visualized on 1.0 % SB gel before Illumina Miseq sequencing at Microsynth (Switzerland).

Reads were trimmed, and quality-checked with FastQC (Babraham Bioinformatics) and FastX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/index.html), and pair-end reads were merged using PEAR (Zhang *et al.* 2013). Clustering was performed with vsearch (Rognes *et al.* 2016) with an initial dereplication step to eliminate all identical reads, and clustering at 97% identity. Chimeras were checked sequentially for de novo, and with the 'gold' database as a reference, with the UCHIME algorithm implemented in vsearch. Finally, all clusters with a size below 5 were removed and reads mapped back at 97 % identity. The final OTU table was converted to BIOM format for downstream analyses. A third of the reads were lost during the clustering process obtaining and a total of 1,243,603 mapped reads belonging to 591 OTUs. The representative OTU sequences were classified against the non-redundant 16S rRNA SSU database in SILVA (v128) with SINA (Pruesse *et al.* 2012). After filtering for chloroplast and mitochondria, a 549 OTU table was obtained that was imported into philoseq object (McMurdie & Holmes 2013) and processed for further analysis. At this point, one replicate of the NH_P+ treatment was discarded due to sample contamination.

In order to study the responses of the widespread OTUs and avoid the stochasticity and the methodological bias error that could constrain the data interpretation (Zhou *et al.* 2011; Ren *et al.* 2017), OTUs appearing at least in the 90 % of the samples were selected for further analysis. The final numerical analyses were performed with 122 OTUs that accounted for 93.3 % of the total OTU sequences and maintained the main traits of the community structure (Fig. S4.2).For more information of taxonomical adscription see Appendix 1.

4.3.4 Numerical analyses

Quantitative differences among treatments were tested by performing one-way ANOVA. The bacterioplankton response to treatments was investigated considering two levels of taxonomic rank: class and OTUat class resolution. The samples were also classified using the UPGMA method. The vegan R package was used (Borcard *et al.* 2011; Oksanen *et al.* 2011).

At the OTU resolution, two complementary approaches were followed. Principal Component Analysis using the Hellinger distance (PCA-Hell) as unconstrained technique (Legendre & Gallagher 2001) and Multivariate Regression Tree (MRT), also using Hellinger distance (Cieslak *et al.* 2012), as constrained technique (De'ath 2002). The MRT provided a hierarchical clustering of response to the different treatments. The best MRT was selected using the Minimal Cross-Validation Relative Error criteria (MCVRE-tree), and further information by the maximum splitting tree (MaxSplit-tree) (Breiman *et al.* 1984; De'ath 2002; De'ath 2007). We used the IndVal index (Dufrene & Legendre 1997) to determine the OTUs characteristic of each cluster at each split level. The procedure compares species fidelity and specificity to groups against randomised species distributions among them. A 999-permutation test was used with no site combination (duleg=TRUE) and the association function "IndVal.g". Only OTUs with p-value < 0.05 were selected as indicators of clusters. We used the R packages mvpart and indicspecies (De Caceres *et al.* 2012). In some specific cases, the Chi-square test ($p<0.01$) was applied to look for significant links between sample clustering obtained from MRT and families of indicator OTUs.

To compare the results obtained in the experiment with the field observations in Zufiaurre *et al.* (submitted-a), we performed a Redundancy Analysis with Hellinger distance (RDA-Hell) to check the distribution of the indicator OTUs in the ENEX experiment throughout the annual cycle of Lake Redon and the changing environmental conditions (Legendre & Gallagher 2001; Borcard *et al.* 2011). All the statistical analyses were performed using R software version 3.2.3 and ggplot2 for plots (Wickham 2009).

4.4 RESULTS

4.4.1 Bacterioplankton abundance

The patterns of bacterioplankton abundance across the experiment were similar for DAPI counts and 16S rRNA copies (Fig. 4.1 A, B); although the absolute values were an order of magnitude lower for the latter. Bacterioplankton was significantly more abundant in the highest P enrichment treatments, about three times more than at low and medium P enrichments ($p < 0.001$ for DAPI and $p = 0.032$ for 16S rRNA). Through the P enrichment gradient there were no significant differences related to the N supply form ($p > 0.099$). In contrast, there was not any significant response to the N imbalance gradient ($p = 0.11$, DAPI; 0.099, 16S rRNA) and also no response to the N source forms ($p = 0.106$, DAPI; 0.39, 16S rRNA).

Assuming that most of the bacterioplankton is heterotrophic in this situation, it is interesting to compare the patterns with indicators of organic carbon availability, DOC

and Chla (Fig. 4.1 C, D). DOC showed no variation among treatments (p = 0.6, P enrichment; p = 0.7 for N imbalance). In contrast, significant differences between P enrichment treatments (p = 0.0001) and the N imbalance, between the lowest and highest N additions (p = 0.008), were found for Chla. There were no significant differences between the N supply forms (p > 0.16). In summary, the bacterioplankton response was similar to the Chla response, although the highest relative increase in the P enrichment gradient occurred at different levels, between the low and intermediate in Chla, and between intermediate and high in the bacterioplankton response.

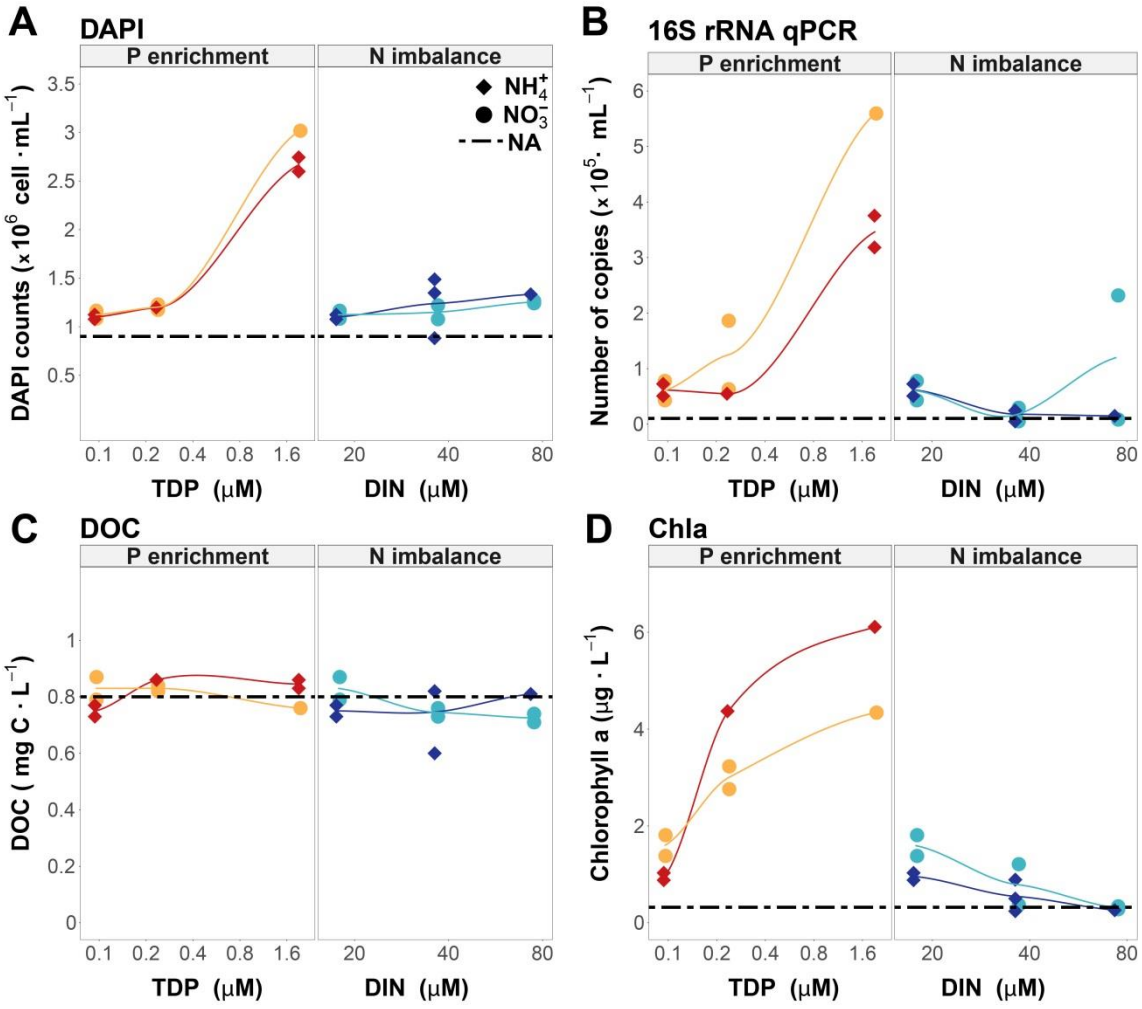


Figure 4.1. DAPI counts (**A**), 16S rRNA copies (**B**), DOC (**C**) and Chla (**D**) values across the experimental gradients. Note the log-scale in the X-axes. Three enrichment levels (low, medium and high) are shown (from left to right) for both P enrichment and N imbalance. The N sources are represented with red or blue diamonds for NH_4^+, and orange or light blue circles for NO_3^-. The average value in the control enclosures (no nutrient addition, NA) is represented by a black dashed line.

4.4.2 The high taxonomic rank response

The relative abundance of the 14 bacterial classes considered did not differ markedly among the diverse treatments (Fig. 4.2 A). However, the hierarchical clustering showed that the differences were consistent according to the treatments (Fig. 4.2 B). The most abundant class was Betaproteobacteria, especially in the no nutrient addition samples (NA), followed by Flavobacteriia and Actinobacteria. Flavobacteriia and Unknown OTUs slightly increased in the P enrichment treatments when NO_3^- was the N supply. Alphaproteobacteria prevailed when NH_4^+ was the N source. Although almost negligible, the relative abundance of Deltaproteobacteria and Cytophagia was higher in the N imbalance treatments in general, and Actinobacteria in the N imbalance enriched with NH_4^+. As could be expected, low nutrient enrichment treatments (HN_P and ON_P) were the most similar to controls (NA).

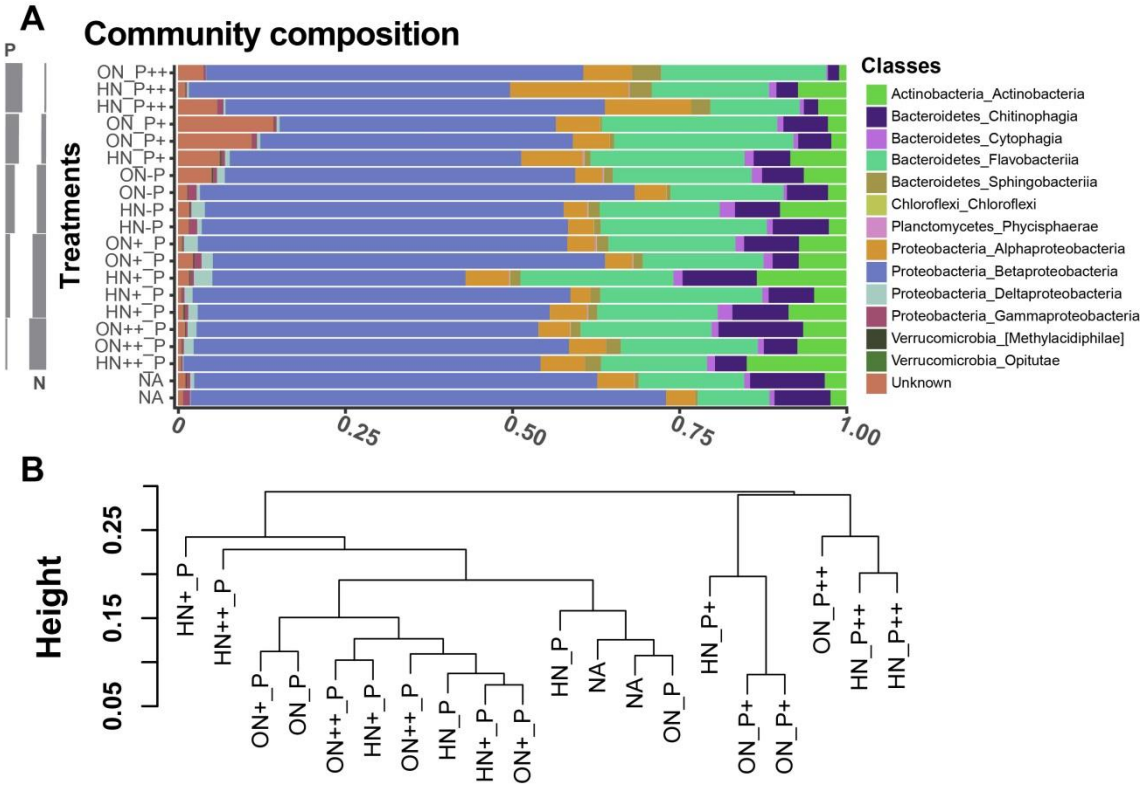


Figure 4.2. (**A**) Relative bacterioplankton composition considering a high taxonomic rank, the class level. The sample order from top to bottom follow the P enrichment in descending order (P++, P+ and P-N), the N imbalance in ascending order (P-N, N+ and N++) and, finally, the no nutrient addition samples (NA). (**B**) Classification of the experiment samples according to the relative abundance of the taxonomic classes using the UPGMA method.

4.4.3 OTU response

The ordination of the samples using the OTU composition also showed similar patterns to those provided by the bacterioplankton abundance. The main community changes were observed along the P enrichment gradient, while N imbalance did not

present major differences regarding NA (Fig. 4.3). Indeed, the first and second axes of the PCA-Hell explained 60 % of the total variation and depicted three main sample groups. The first axis distinguished the P enrichment from the N imbalance and NA samples. The second axis split the medium (N_P+) and high (N_P++) P enrichments. A few OTUs showed a strong loading on the axes. Specifically, OTUs 58 and 80 were associated with N_P++; OTUs 19 and 47 to N_P+ and the OTUs 1, 11 and 26 defined the poor P treatments.

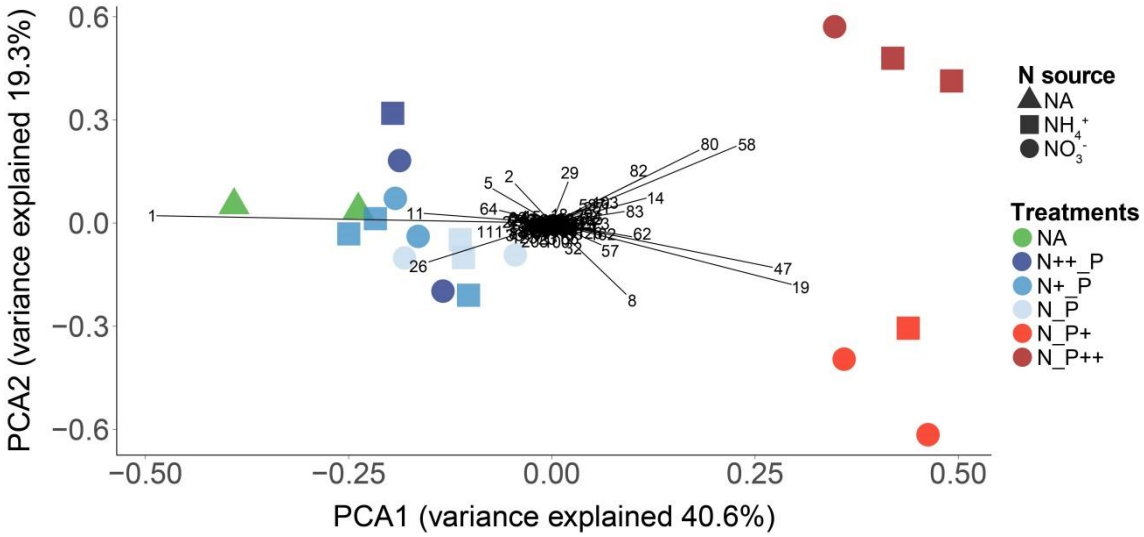


Figure 4.3. Ordination (PCA-Hellinger) of the experimental treatments based on the relative abundance of the 122 OTUs that were present in 90% of the samples.

The sample classification using the MCVRE-tree reinforced the response structure indicated in the PCA-Hell and provided some more details (Fig. 4.4). The third split into four classes was the optimum classification, including 61 % of the total variation. Further significant divisions (MaxSplit-tree) did not improve the classification significantly (Fig. 4.4). The first two splits already accounted for the structure found in the PCA-Hell. The third differentiated the controls (NA) from the N imbalance treatments. The rest of the trees accounted for subtleties within the N imbalance gradient, mostly differentiating the treatment with high NH_4^+.

The number of indicator OTUs associated with each split declined as the tree splitting increased. If we consider the optimum third split, the MCVRE-tree presented a total of 14 indicator OTUs related to N_P++ enrichment, 17 to N_P+, 15 to N imbalance and 11 for NA. Overall, P enrichment treatment obtained the highest number of indicator OTUs, with 51 % of the total indicator OTUs related to N_P+ or N_P++ treatments, 31 % to N imbalance and 18 % to the NA samples.

In general terms, there was no conserved response of high taxonomical ranks to specific treatment cluster. Single treatment clusters were represented by OTUs from different classes, and those taxonomical classes were represented in several treatments. Alpha- and Betaproteobacteria were the most represented classes among the indicator OTUs, encompassing both the 50 % of the total indicator OTUs (Fig. 4.4). Within Betaproteobacteria, Comamonadaceae and Oxalobacteriaceae were the most characteristic families. The relatedness of Alphaproteobacteria (Rhodobacterales, Sphingomonadales, Rhodospirillales and Rickettsiales) to P was high, with 80 % of indicators in P enrichment.

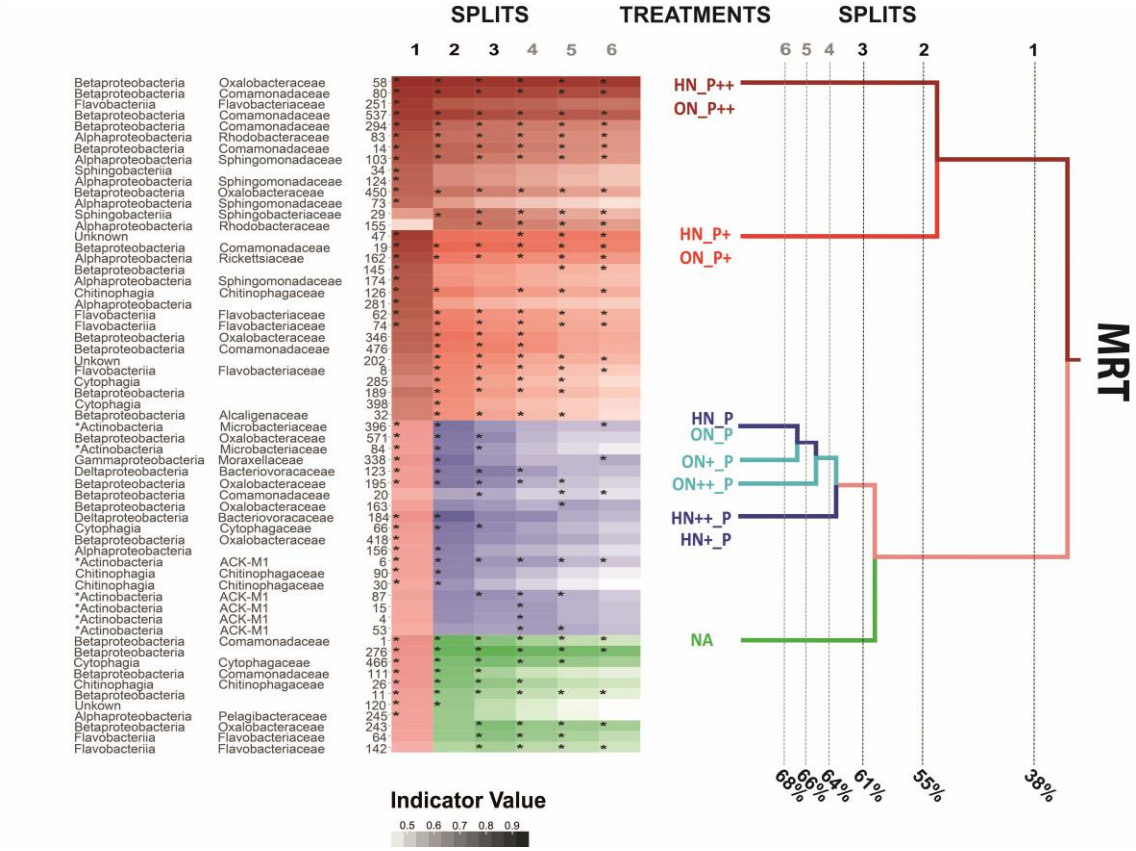


Figure 4.4. Classification of the experimental samples using MRT and indicator OTUs determined by IndVal analysis. The variance explained associated with each MCVRE-tree split is indicated with dashed lines. The first three splits are highlighted as indicative of the best classification, although up to six were significant. The IndVal analysis is represented by a heatmap, and the colours correspond to the branches in the MRT. The darker the colour, the higher the IndVal value. The class, family and number of the indicator OTUs are listed in the left columns. The "*" in the heatmap represents a p-value<0.05 in the IndVal significance at each split level. The "*" in the taxon list indicates the non-random distribution of the Actinobacteria among clusters (p-value <0.01 at Chi-square test).

The indicators of N imbalance enriched with NO_3^- belonged to Beta- (Comamonadaceae and Oxalobacteriaceae), Delta- and Gammaproteobacteria (Bacterivoraceae and Moraxaceae). The unique case when Proteobacteria were not the

main indicators was in N imbalance enriched with NH_4^+. In this case, the ACK-M1 family from Actinobacteria became the most representative group, with 45 % of the indicator OTUs. In fact, Actinobacteria was the only class significantly correlated to a treatment cluster (Chi-square test, p-value <0.01), specifically, the N imbalance enriched with NH_4^+.

4.4.4 Annual distribution of P and N indicator OTUs

The indicator OTUs covered a wide range of occurrence and abundance patterns within the annual cycle of Lake Redon (Zufiaurre *et al.* submitted-a) (Fig. 4.5). There were since ubiquitous organisms that appeared every month at all sampled depths (e.g., OTUs 4, 47, 11, 19) to OTUs restricted to a few samples (e.g., OTU 338 was found 3 times). Similarly happened with abundance, OTU 4 was the most abundant ($1 \cdot x\ 10^5$ copies) among any OTU found during the annual survey and OTU 338 one of the less (10 copies). In general, most of the indicator OTUs can be qualified of common and abundant in the bacterioplankton community of Lake Redon. Therefore, it made sense to compare the indicator character found during the experiment with the environmental conditions in which the OTUs proliferate during the annual lake cycle.

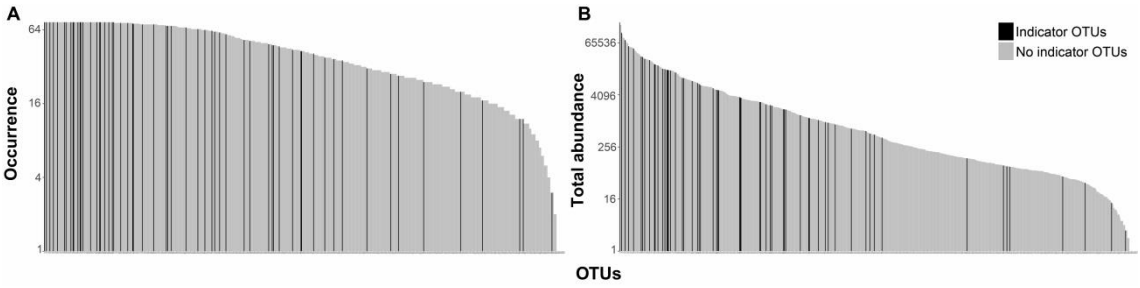


Figure 4.5. Ranked occurrence (**A**) and total abundance (**B**) of the indicator OTUs (black) compared to the 700 OTUs (grey) found in the annual cycle of Lake Redon. Note the logarithmic scales.

The main difference between the resource conditions in the experiment and those in the lake throughout the year is the change in the correlations between carbon, nitrogen and phosphorus sources. In the experiment, because of the artificial fertilization, there was a high correlation between Chla and TDP, whereas in the lake cycle there was no such correlation between them (Fig. 4.6) as when dissolved phosphorus becomes available it is quickly uptaken by phytoplankton and TDP only accumulates during winter when no light penetrates the snow and ice cover. On the other hand, in the experiment, the relative availability of NO_3^- and NH_4^+ were negatively correlated because of the experimental design. However, in the natural lake cycle, these compounds tend to change similarly and in an opposed way to Chla. Finally, the

variation of DOC across treatments was low, whereas in nature, there was a correlation between Chla and DOC, since the external source of DOC in Lake Redon is low. This contrasting structure of correlations between the experiment and the field conditions might help in clarifying whether the bacterioplankton responses were direct to the nutrient additions or mediated by the effects that the additions had on phytoplankton and planktonic food-web in general.

The constrained ordination (RDA-Hell) performed using the abundance distribution of the indicator OTUs along the annual cycle depicted the seasonal cycle (Fig. 4.6). The first axis highlighted the effect of productivity increase during the deepening of the thermocline from August to October at 2 m, 10 m and 20 m, indicated by higher Chla and DOC. OTUs related to this event were indicators of P enrichment in the enclosure experiment, namely, OTUs 202, 8, 126, 19, and 281 from N_P+ and OTUs 29, 251, and 14 from N_P++. Most of them belonged to the Bacteroidetes phylum (Appendix 1). On the opposite side of the axis, NH4+ was the weightiest environmental variable. The

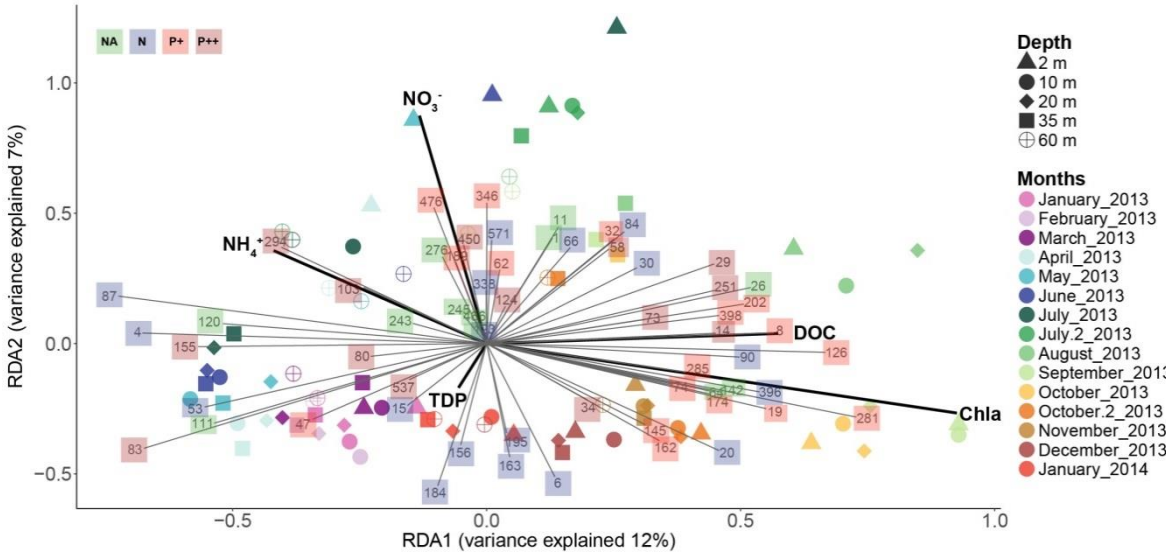


Figure 4.6. Biplot of the Redundancy Analysis (RDA-Hell) of the monthly distribution of the indicator OTUs identified in the enclosure experiment based on (Zufiaurre et al. submitted-a). The monthly distribution of the samples is highlighted in different colours and the depths by the symbol shape. The black arrows depict the environmental variables and the grey ones the OTUs. Coloured squares of each OTU represent the enrichment they indicate in the experiment.

extreme mainly included spring samples from April to early July, which correspond to the late ice-cover, thawing and early ice-free periods. Most relevant indicator OTUs associated with this part of the axis belonged to the family ACK-M1 of Actinobacteria (OTU 87, 4 and 53), which were indicators of N imbalance with NH_4^+ as N source in the experiment (Fig. 4.4). Nevertheless, some OTUs related to treatments N_P++ also loaded in this direction (i.e., OTUs 83, 80 and 155).

The RDA second axis was defined by a gradient between NO_3^- and TDP. The high TDP- low NO_3^- conditions were associated with the winter ice-covered period. The early ice-cover phase (December, January) was associated with indicators of NH_4^+ (OTUs 15, 6, 184 and 156). According to this result, the experimental response to high NH_4^+ could be related to the relative concentration respect NO_3^- rather than directly to NH_4^+. In contrast, the mid-ice-cover period (February, March) was associated with indicators of P enrichment in the experiment (OTUs 83, 80, 537 and 47). The other extreme of this second axis, related to high NO_3^- included samples from the surface at the end of the ice-covered period and spring mixing (from May to July between 2 to 35 m depth). However, the OTUs loading in this direction was a mixture of indicator of all the clusters.

4.4.4 OTU idiosyncratic response

OTUs belonging to Comamonadaceae, the most abundant family in the experiment, presented a wide variety of responses to the treatments (Fig. 4.7). Indeed, a PCA-Hell performed exclusively with the OTUs of this family (Fig. S4.2 A) mostly reproduced the patterns showed by the whole OTUs set (Fig. 4.3). OTU 80 increased at N_P++ treatments (Fig. 4.7 A) and OTU 19 at N_P+ (Fig. 4.7 B). Other OTUs increased their abundances at the N imbalance treatments, such as OTU 20 (Fig. 4.7 D), or reacted negatively to enrichments as OTU 1 (Fig. 4.7 C) that, consequently, was an indicator of the NA samples (Fig. 4.4). These four OTUs also were related to contrasting environmental conditions and periods of the year in the lake survey (Fig. 4.6).

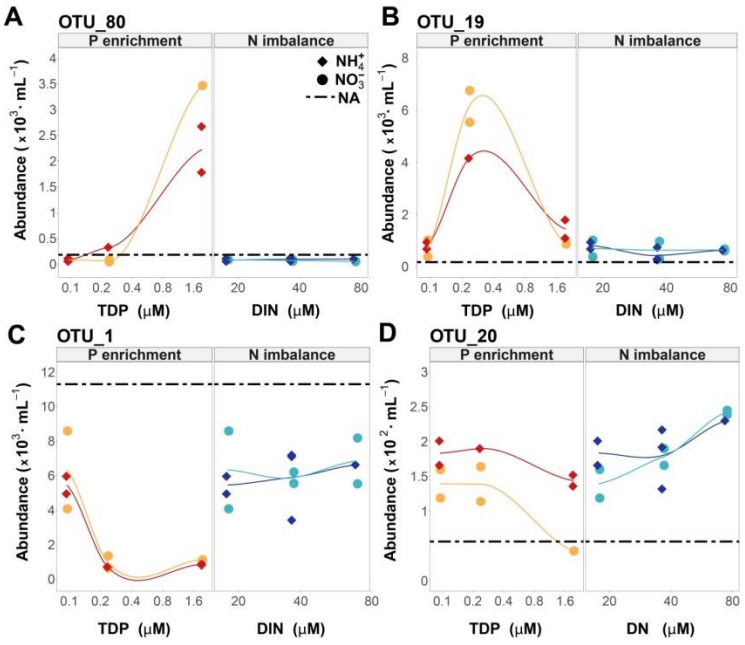


Figure 4.7. Response to nutrient addition of some representative OTUs of the Comamonadaceae family. Note the log-scale in the X-axes. Three enrichment levels (low, medium and high) are shown (from left to right) for both P enrichment and N imbalance. The N sources are represented with red or blue diamonds for NH_4^+, and orange or light blue circles for NO_3^-. The average value in the control enclosures (no nutrient addition, NA) is represented by a black dashed line.

The lack of a coherent response of OTUs from the same family also occurred with other families. In the Oxalobacteriaceae, also an abundant family, only one OTU (58) responded to N_P++ (Fig. S4.2 B). Within Actinobacteria, OTU 6 was favoured under N imbalance conditions, especially by NH_4^+ (Fig. S4.3 A), while OTU 396, was negatively affected by both enrichments (Fig. S4.3 B). Other OTUs grew in both P and N enrichment gradients (OTU 29, Sphingobacteriia, Fig. S4.3 C) or, conversely, negatively affected by both the enrichments (OTU 5, Comamonadaceae, Fig. S4.3 D). In summary, there were many OTUs which response patterns differed from the main bulk bacterioplankton response even among phylogenetically close organisms.

4.5 DISCUSSION

4.5.1 The effects of P enrichment

The experimental P addition resulted in a phytoplankton increase as expected in a P-limited lake. The bacterial abundance followed similar tendencies, although not identical. There was a great number of OTUs significantly reacting to the P addition. Most of them belonged to Betaproteobacteria, an abundant class in freshwater ecosystems, known to present opportunistic growth and increase with nutrient availability (Salcher *et al.* 2008; Newton *et al.* 2011). In particular, the families Comamonadaceae and Oxalobacteriaceae were the most abundant families in the experiment and included many indicator OTUs of P enrichment. Alphaproteobacteria are less common in freshwaters than Betaproteobacteria (Newton *et al.* 2011); nevertheless, they were the second group, with most indicator OTUs of P enrichment. When the results of the experiment were compared with the annual cycle in the lake, two kinds of response to the P enrichments could be distinguished. On the one hand, most of the P indicator OTUs were related to the productive peaks in the annual cycle, in which Chla and DOC increase. There is more available labile carbon for heterotrophic bacteria during these periods. The OTUs mainly belonged to the phyla Bacteroidetes and Alphaproteobacteria. Bacteroidetes are mainly copiotrophs and good in degrading organic compounds (Fierer *et al.* 2007; Newton *et al.* 2011). At the family level, Flavobacteriaceae were the most common, followed by Cytophagaceae and Sphingobacteriaceae; all of them related to organotrophic metabolisms (Oren 2014). In contrast, the Alphaproteobacteria most represented were Rickettsiales, a lineage characterized by a reduced genome and a parasitic life form (McCutcheon & Moran 2012). On the other hand, there were some P indicator OTUs in the experiment that did associated with periods of higher P availability but not associated with phytoplankton growth because of the lack of light. These OTUs may be responding directly to P water availability and included members of the Comamonadaceae (Betaproteobacteria), Rhodobacteraceae (Alphaproteobacteria) and some OTUs of unknown affiliation. If we

focus in the four OTUs that responded strongly to P enrichments, either to N_P++ treatments (OTUs 58 (Oxalobacteriaceae) and 80 (Comamonadaceae)) or N_P+ (19 (Comamonadaceae) and 47 (Unknown)), in each case, there was one responding directly (80 and 47) and one indirectly (58 and 19). These might be viewed as a resource partitioning among species that may facilitate coexistence (Chesson 2000; Alexander *et al.* 2015); particularly, in the case of the phylogenetically close Comamonadaceae OTUs.

4.5.2 N imbalance and N supply forms

The bacterial increase to N additions was modest compared to the P enrichments. However, there was a clear differentiation when the source was ammonium or nitrate. All indicator OTUs from Actinobacteria were related to the N imbalance gradient: two OTUs from Microbacteriaceae to low N additions and five from ACK-M1 to NH_4^+. A close relationship between Actinobacteria ACK-M1 and NH_4^+ supply has been previously reported (Newton *et al.* 2011; Dennis *et al.* 2013). During the Lake Redon annual cycle most of these ACK-M1 indicator OTUs appeared related to periods of low DOC and Chla and high NH_4^+, a situation occurring mainly in spring and typical of the end of the ice-cover period and thawing before water column complete mixing and phytoplankton blooming (Catalan 1992a). One of the ACK-M1 OTUs (4) was the most abundant OTU during the annual cycle and played a background role with stable density across depths and time (Zufiaurre *et al.* submitted-a). Finally, ACK-M1 OTU 6 behaved differently from the other indicators of the family during the annual cycle; it peaked at low NO_3^- in December. ACK-M1 is among the most abundant Actinobacteria families in freshwater ecosystems (Lindström *et al.* 2005; Jezbera *et al.* 2009) characterized by its tendency for oligotrophic conditions (Garcia *et al.* 2014). From our results, it appears that ACK-M1 may also prefer, at least some OTUs, a high imbalanced N:C ratio in terms of resources. The two Microbacteriaceae OTUs indicative of low N imbalance treatments appeared related to the ice-free period during summer stratification and to the deep chlorophyll maxima, which do correspond to situations of low NH_4^+. Similarly, the NO_3^- indicator OTUs during the experiment (Moraxellaceae, Oxalobacteriaceae, *Limnohabitans* (Comamonadaceae)) were not associated with periods of high nitrate during the annual cycle; they proliferated mostly during the autumn overturn and early ice-cover period, situations of low NH_4^+ and also NO_3^-. They seem to respond to an opposed N:C ratio than the observed for the ACK-M1. The only exception was OTU 571 (Oxalobacteriaceae) that was associated with deep layers at the end of the ice-covered period where NO_3^- increase by nitrification (Catalan 1992a) and a complex and rich bacterial community develops (Zufiaurre *et al.* submitted-a).

4.5.3 Coexistence and niche differentiation

Lake Redon is a deep ultraoligotrophic lake. The upper 50 m are well-oxygenated throughout the year, and nutrients and organic carbon are low. Within the wide range of environmental variation in freshwater ecosystems, it can be view as a relatively homogenous environment favouring certain functional bacterial guilds, which can be reflected at high bacterial taxonomic ranks (Philippot *et al.* 2010; Morrissey *et al.* 2016). However, the coexistence of functionally closed species competing for the same resources requires some degree of niche differentiation and environmental fluctuation, so that the differences in relative fitness, which eventually will bring to a unique competitor winner, are compensated by the niche differentiation (Chesson 2018). Therefore, at some point, OTUs of the same high bacterial taxonomic rank have to differ in their niche. Despite the high oligotrophic conditions, nutrient fluctuations occur throughout the year, causing changes in primary production and, thus, the availability of carbon substrates. It could be expected some niche differentiation of phylogenetically close OTUs across this variation. In our results, Betaproteobacteria provide an excellent example of potential coexistence by resource partitioning (HilleRisLambers *et al.* 2012). The group showed indicator OTUs across the clustering tree differentiating the treatments response. Even at lower taxonomic rank, OTUs of the families Comamonadaceae and Oxalobacteriaceae spread throughout the treatment clusters as indicators, from high P additions to controls. Quantitatively, this idiosyncratic character of the OTUs was particularly evident in the PCA-Hell of the experiment. The extremes of the first and second axes were mainly defined by three Comamonadaceae species (OTUs 1, 80 and 19). Similar features were shown by other groups (e.g. Flavobacteriia). The exception was Actinobacteria, and more specifically, the ACK-M1 family. In this case, all indicator OTUs concentrated in the NH_4^+ enriched treatments and, many of them showed relative dominance in similar conditions during the annual lake cycle. Interestingly, the absolute populations in the lake were quite stable (i.e., OTU 4, in particular). In this ACK-M1 family, the source of long-term stability seems to be different from resource partitioning — at least at the gross view of nutrient availability here addressed. Predation may also stabilize coexistence (Chesson & Kuang 2008). Some authors report Actinobacteria positive selection trend by a number of phagotrophic flagellate species (Ballen-Segura 2012; Gerea *et al.* 2012). Differences in the degree of susceptibility to a common predator may result in stabilizing niche differentiation.

4.6 SUPPORTING INFORMATION

Taula S4.1: Primers and thermal cycling details for the 16S rRNA qPCR and Illumina sequencing

Target	Genes Primer names	Sequences (5'-3')	Thermal cycling	Technique	References
Bacteria 16S rRNA	**16S rRNA** 341F 534R	 CCT ACG GGA GGC AGC AG ATT ACC GCG GCT GCT GGC A	(95°C, 7min)x1 (95°C, 15s; 60°C 30s; 72°C, 30s; 80°C, 10s)x35 (95°C, 15s;(60 to 95°C, 10s increment 0.5°))x1	qPCR	López-Gutierrez *et al.*, 2004
Prokaryote 16S rRNA	**16S rRNA** Pro341F Pro805R	 AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGC-TCTTCCGATCTCCTACGGGAGGCAGCAG**CCTACGGGNBGCASCAG** CAAGCAGAAGACGGCATACGAGATNNNNNNGTGACTGGAGTTCA-GACGTGTGCTCTTCCGATCT**GACTACNVGGGTATCTAATCC**	(98°C, 3 min)x1 (98°C,30s; 55°C,, 30s; 72°C, 45s)x8 (72°C, 5min)x1	PCR for NGS	Takahashi *et al.*, 2014

Figure S4.1: Abundance distribution of the main bacterial groups considering the whole data set and after the selection of OTUs present in 90% of the treatments.

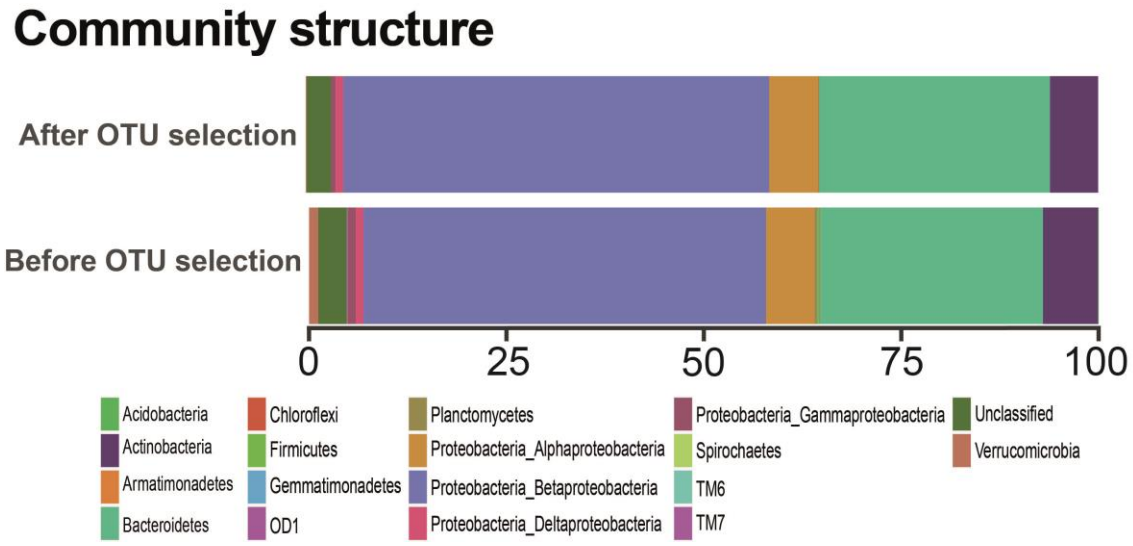


Figure S4.2: Principal Component Analyses PCA using the Hellinger distance (PCA-Hell) of the OTUs belonging to the Comamonadaceae (A) and Oxalobacteriaceae (B) families (Betaproteobacteria).

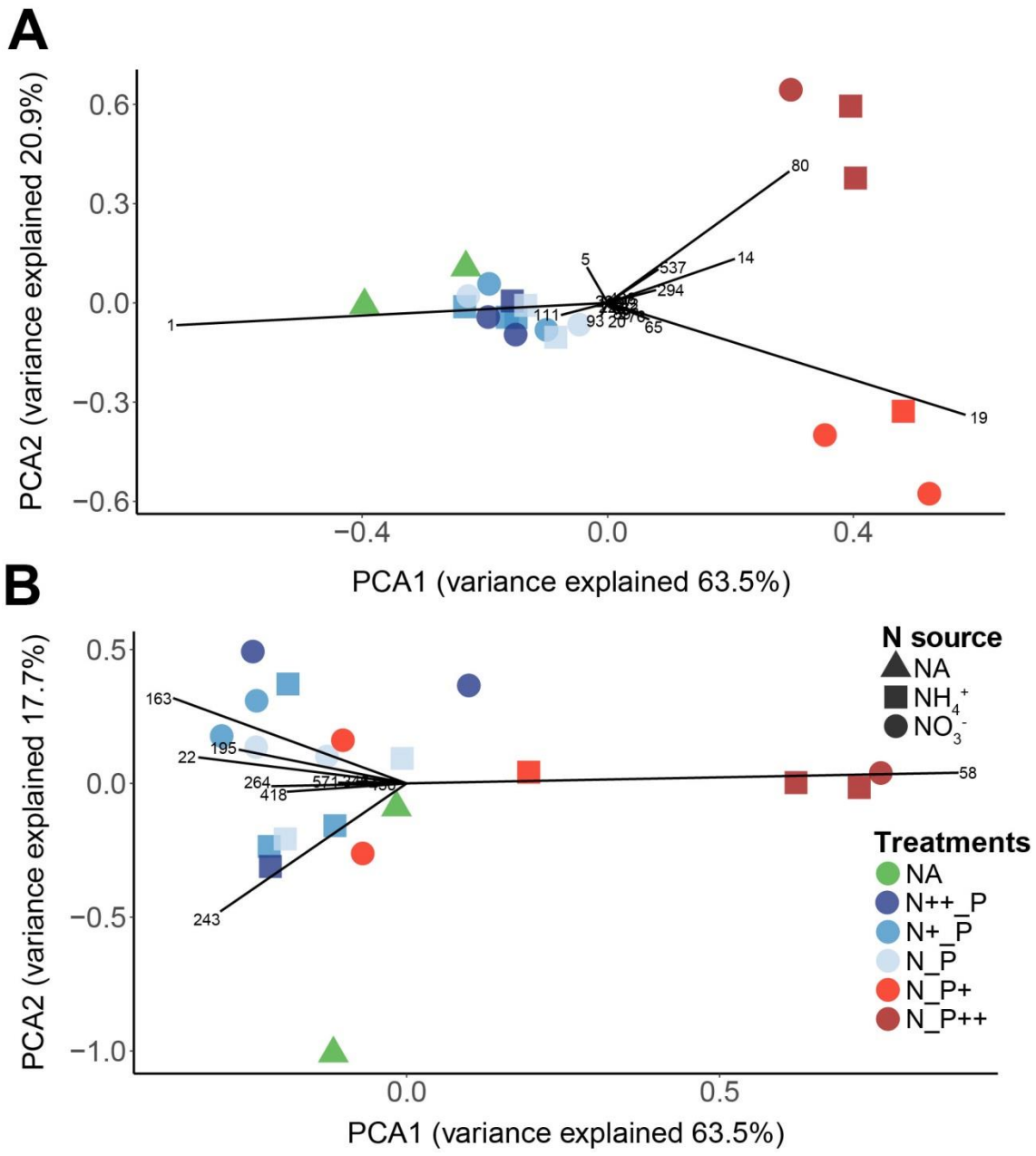
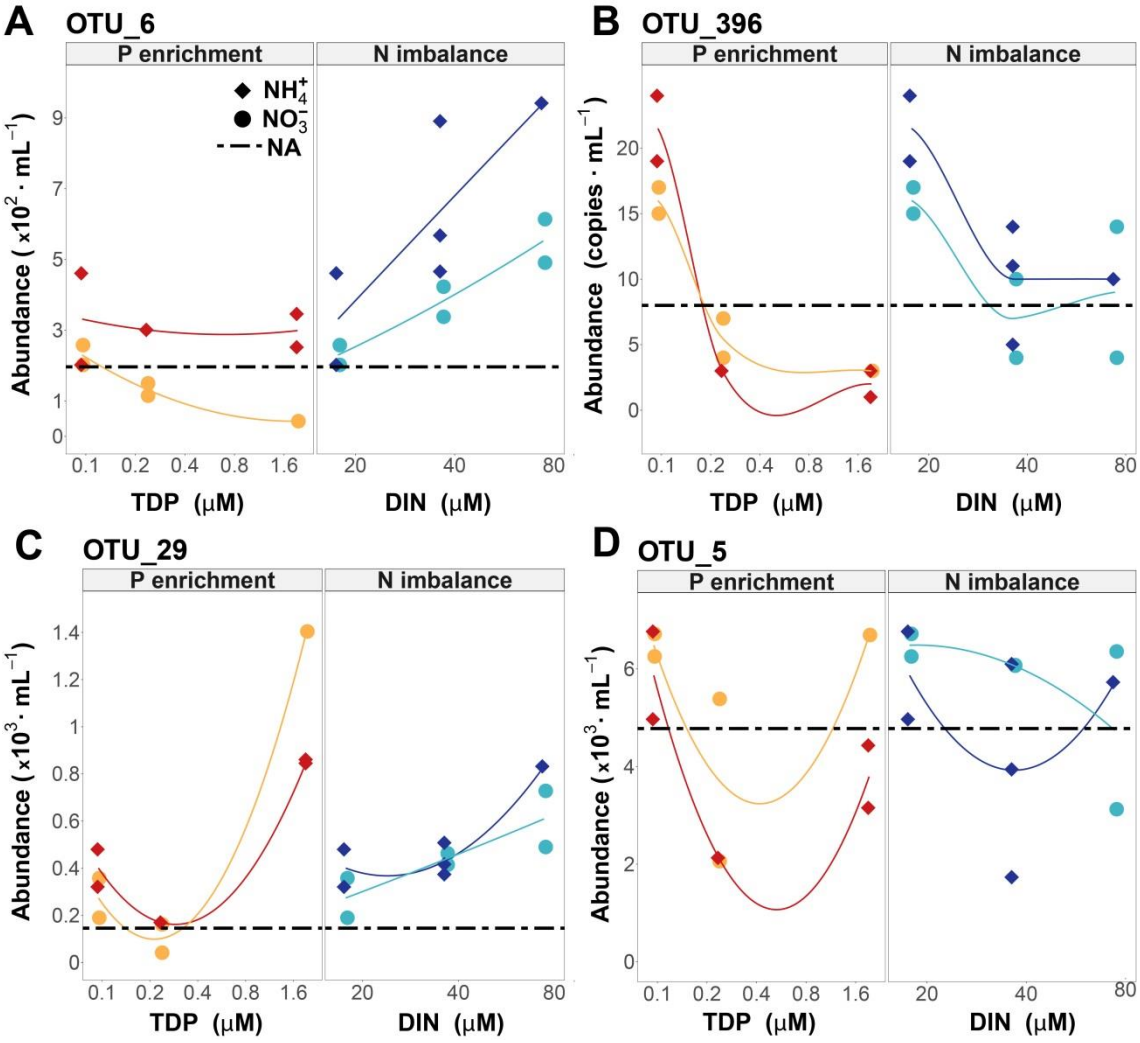


Figure S4.3: Examples of the diversity of responses to the experimental treatments at the OTU level. Three enrichment levels (low, medium and high) are shown (from left to right) for both P enrichment and N imbalance. Note the log-scale in the X-axes. The N sources are represented with red or blue diamonds for NH_4^+, and orange or light blue circles for NO_3^-. The average value in the control enclosures (no nutrient addition, NA) is represented by a black dashed line.



The top of the mountain is always
the bottom of another

-Marianne Williamson-

CHAPTER 5
Conclusions

5.1 Wrap up

Although periods of significant environmental changes are part of Earth dynamics, since the industrial revolution not only climatic seasonality is being hastily modified; but the biogeochemical cycles are suffering drastic changes (Steffen *et al.* 2015). Such pronounced environmental changes affect biologic diversity at all trophic levels, from the minuscule organisms to predators at the top of the trophic chain. Despite their tiny size, microorganisms play a major role in nutrient cycling, ecosystem functioning and energy transfer through the trophic chain (Cotner & Biddanda 2002), being thus in the frontline facing climatic changes.

This thesis aimed to contribute to the current knowledge of environmental fluctuations effects over microbial communities. There were two focuses: First, the seasonal dynamics and strategies of the bacterioplankton community facing extreme seasonal changes in a seasonally frozen oligotrophic lake; and second, the effect of episodic nutrient loads on protist and bacterioplankton communities, focusing on low taxonomic rank levels.

5.1.1 Bacterioplankton seasonal changes

Understanding seasonal community fluctuations have always been a major goal in ecology. Although assemblages may not be annually identical, long-term seasonal studies of microbial plankton communities have shown reoccurring patterns (Berman *et al.* 1992; Fuhrman *et al.* 2006; Crump *et al.* 2009). Protist diversity and community fluctuations were earlier characterized due to the easier species identification with conventional microscopic techniques, therefore their seasonal fluctuations are thoroughly described. Insights into the bacterioplankton community were technically limited, and it was not until the development of molecular techniques that the huge diversity of bacterioplankton started to be assessed (Pedrós-Alió 2012). However, detailed data about bacterioplankton diversity in seasonally ice-covered lakes is still limited.

In seasonally ice-covered ecosystems, attention has been mainly placed in the ice-free season (Bertilsson *et al.* 2013; Powers & Hampton 2016; Hampton *et al.* 2017). In chapter 2, the balanced sampling effort throughout the year in a lake with a half a year ice cover is described, and the results showed that the winter period rather than epilimnion during summer constitute the fundamental period determining bacterioplankton composition.

As a new paradigm, the results suggest that there are three different types of bacterial assemblages: resistant, resilient and opportunistic. There is a core of bacterioplankton assemblages -fundamentally developing at low irradiance- that become resistant and resilient to seasonal changes in contrast to the expected seasonal community replacement patterns described in former studies (Crump *et al.* 2009; Cruaud *et al.* 2019). Added to this core, there are opportunistic assemblages that bloom at specific seasonal periods such us summer epilimnetic waters.

Background OTUs, which changes, across depth and time, were not significantly different from random fluctuations, define the resistant assemblage. The IndVal analysis (De Cáceres & Legendre 2009) was a useful procedure to detect them. It was a relevant part of the community since gathered some of the most abundant OTUs and in some periods represented the third part of the total community abundance. Background assemblages comprise organisms highly tolerant to environmental fluctuations and physiologically flexible (Allison & Martiny 2008).

In contrast to the expected greater bacterioplankton diversity during the ice-free season as the phytocentric view would dictate, there was higher diversity and a greater number of indicator OTUs during the winter period. The results suggest that bacterioplankton and phytoplankton community dynamics are not exactly coupled. Both communities match in terms of productivity, as long as the highest bacterioplankton abundances were recorded in the hypolimnion during the ice-free season linked to the greater phytoplankton abundances at the upper hypolimnion during the summer stratification (Figure 2.1 and 2.2). Conversely to phytoplankton, bacterial diversity was higher at low light conditions, either under-ice or in deep summer waters. The assemblages of the two ice-covered periods sampled were connected by the hypolimnion during summer stratification, showing community persistence in deeper layers. The large depth of lake Redon likely favoured the resilience of the under-ice assemblage providing refuge against the harsh conditions (high temperatures and high radiation) of the epilimnion during the ice-free season.

Epilimnion during summer has been the target in most studies of seasonally ice-covered lakes (Ortiz-Álvarez *et al.* 2019). Nonetheless, the present study has shown that bacterioplankton diversity impoverished in summer shallow layers, suggesting harmful conditions for many OTUs (Figure 2.5). The adverse effect that high UV radiations arriving at the epilimnetic layers of the high mountain lakes has over the growth of several bacterial lineages would be one of the main drivers of such diversity decline (Sommaruga *et al.* 1999; Sommaruga 2001; Sarmento *et al.* 2015). Only some OTUs opportunistically took advantage of these harsh conditions to proliferate.

The great depth and long water resident time of Lake Redon likely contributed to the stability of the bacterioplankton core community. In shallower lakes, washout and the

lack of a summer refuge may imply a rather different dynamics, where perhaps recolonizations must be more relevant. On the other hand, if ice cover duration tends to shorten — as expected from on-going global trends— the resilience and resistance of the core assemblage could be jeopardized even in large lakes, and a sudden shift towards a different characteristic community may occur. Likely, the opportunistic assemblage, particularly adapted to actual epilimnetic conditions, would gain overall relevance and expand as conditions throughout the year become more suitable

5.1.2 Episodic nutrient enrichments and microbial plankton diversity

Nutrient additions have been traditionally used to reveal fitness differences among co-occurring autotrophic species. In the ENEX experiment, gradients of nutrients were established to disentangle between relative fitness and niche preferences among species. Besides, different N sources provided insights into niche differentiation patterns between phylogenetically related species.

In Lake Redon, both protist and bacterioplankton growth increased with P enrichment, and not with N addition. A recent study indicated a decrease of water column DIN concentrations in Lake Redon due to the increased P deposition during the last decades (Camarero & Catalan 2012). Nonetheless, the N depletion has not been large enough to revert conditions to N limitation or co-limitation, otherwise analogous patterns to P enrichment would have been found in N imbalance gradient. Thus, our results indicate that if P deposition continues rising, lake productivity may still increase as described in other mountain ranges (Reche *et al.* 2009; Brahney *et al.* 2015).

In any case, the P gradient was the playground for competition and relative fitness differences. There was a phylogenetic clustering of the main taxonomic groups across the P axis. Chrysophytes prevailed at low and medium P additions, likely related to their competitive ability, while cryptophytes, diatoms and chlorophytes at high P, following their nutrient requirements (Phillips *et al.* 2013). Such clustering of main taxonomic groups across P gradient indicates a phylogenetically conserved niche regarding P availability. N becomes a niche stabilizing mechanism in different ways. On the one hand, N imbalance favoured the coexistence of major groups, as the oligotrophy adapted dinoflagellates and chrysophytes, due to their differential sensibility to N concentrations. On the other hand, the different N use was the key for resource partitioning in P enrichment. In agreement with a recent study that pointed towards a differentiated N source preference among algae (Glibert *et al.* 2016), we found that phylogenetically close related species segregated according to different N source with the same P availability. Hence, belonging to different N-use guilds is a

form of resource partitioning that contributes to niche differentiation and thus coexistence in a fluctuating environment.

Whether species were indicators or not, maximum growth rates were higher in species with initially lower densities. This frequency-dependence of growth can be interpreted as intraspecific competition exceeding the interspecific competition and thus, the larger the population, the more the species limit their own growth rate by internal competition (Chesson 2018; Gallego *et al.* 2019). The similar response to additions of autotrophic and heterotrophic protists may be indicative of strong direct and indirect interactions that may enhance niche differentiation and coexistence.

Commonly, theoretical and modelling approaches have been used to approximate the coexistence of different planktonic species (Kemp & Mitsch 1979; Schippers *et al.* 2001; Scheffer *et al.* 2003; Kenitz *et al.* 2013; Huang *et al.* 2016). Experiments are rare and frequently limited to a few species (Alexander *et al.* 2015; Jewson *et al.* 2015). Here, we provide an in situ experiment where an entire community was targeted to identify the mechanisms of species coexistence. Overall, our results showed that there is a negative correlation between relative fitness and niche differentiation of the species, the greater the fitness, the lower the need to differ the niche. The coexistence of planktonic protist species is more complex than pairwise species interaction. Strong and weak networks between species contribute to coexistence, and more than a paradox, the coexistence of many species with similar nutrient requirements should be understood in a context were small differences in resources between similar species allow their coexistence.

The bacterioplankton assemblages changes through the ENEX experiment were the result of both the direct effect of nutrient additions and indirect effect related to protist community response. P was the main driver for the bacterial biomass. Nonetheless, OTUs directly responding to P were only a few as assessed by the comparison with the annual cycle in Lake Redon. Most of the P enrichment indicator OTUs were responding to the increased labile carbon for bacteria produced by enhanced phytoplankton growth.

Indicators of direct P effect were OTUs related to low productive periods that showed low abundances throughout the year, and that only increased significantly in the enclosures when P was added (Figure 5.1, OTU 80). On the contrary, indirect P effect indicators were OTUs related to more productive moments of the lake such as the overturn periods (Figure 5.1, OTU 32 and OTU 14), as well as OTUs from the summer epilimnetic waters (Figure 5.1, OTU 19 and OTU 8). The latter belong to the opportunistic assemblage, and their growth was clearly favoured in the enclosures with high P availability. Some indicator OTUs of N imbalance were associated with overturning periods (Figure 5.1 OTU 84). The experimental conditions had a negative effect over certain taxa; one clear example was the ubiquitous and abundant OTU 4

that belongs to the resistance assemblage defined by the annual cycle. In the ENEX experiment, this OTU was an indicator of N imbalance, but not because N rich conditions favoured its growth, but rather the negative effect was weaker in the N imbalance than in P enrichment gradient (Figure 5.1 OTU 4). Because the nutrient conditions in the experiment were not exceptionally different than during the annual cycle (except for the highest additions), the decline of OTU_4 in the experiment should be attributed to the particular conditions in the enclosures, perhaps related to facilitated grazing conditions. Overall, similar to the protist community, the bacterioplankton of the lake Redon must require some degree of niche differentiation to coexist. However, in contrast to protists that showed high taxonomic rank clustering regarding the P gradient, this was not the case for bacterial classes. However, lower taxonomic rank categories, i.e. families, did show OTUs segregation across the nutrient treatments indicating niche differentiation within the families. An exception was the Actinobacteria ACK-M1 family that showed clustering at moderate ammonium additions.

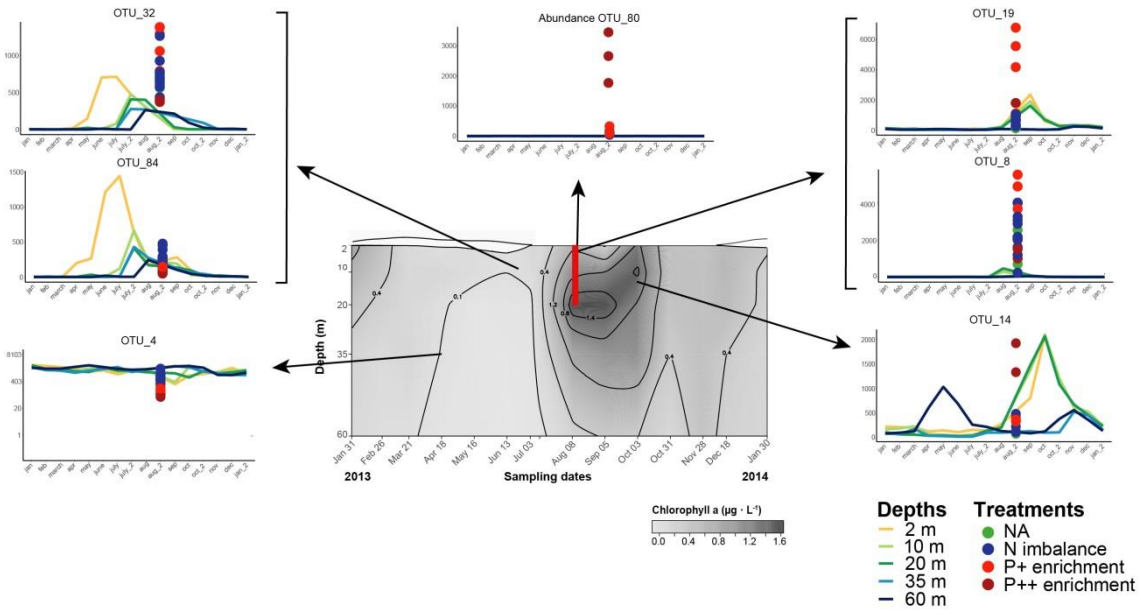


Figure 5.1. Seasonal and enclosure abundances of some ENEX experiment indicator OTUs: OTU 34, OTU 84, OTU 4, OTU 80, OTU 19, OTU 8 and OTU 14. Density plot depicts chlorophyll-a concentrations along the studied period in chapter 2, and the red line marked depth and date of the enclosure experiment.

5.1.3 The hidden (stable) microbial planktonic community

The great number of recovered taxa from an epilimnetic sample was one of the highlighting results of the ENEX experiment. The epilimnion in high mountain lakes is considered a nutrient and species-poor environment (Felip & Catalan 2000). Nonetheless, in the enclosures, we recorded almost all the protist species found in former studies in Lake Redon (Felip *et al.* 1999a; Felip *et al.* 1999b). Including species

typical from the hypolimnion and rare ciliates previously observed only in the slush layer during the winter period. These rare ciliates appeared in high P enriched with ammonium as N supply treatments. The most similar nutrient conditions to the slush layer. Similarly, species corresponding to the hypolimnion also were recovered in the P enrichment treatments. The hypolimnion during summer stratification is richer in nutrient than the hypolimnion, and light environment within the enclosures may be similar to that in the upper hypolimnion.

In the case of prokaryotes, no previous data about the seasonality of the lake Redon was available until the confection of this thesis. Almost all the bacterioplankton OTUs found in the enclosures were also encountered during the annual lake cycle, indicating those OTUs were typical of the lake bacterioplankton community and not an artefact of the enclosures. Almost all the ENEX indicator OTUs were found in the water column at the moment of the enclosure settlement; nonetheless, the abundances within the enclosures were in general far from the abundances found in the epilimnion.

The results of the experiment indicate a high persistence of the microbial species in the water column. There is a permanent hidden community that may respond to a long-term stable non-equilibrium coexistence of species. Remaining in low abundance or as resting stage are mechanisms that prevent competition and enhances buffered population growth and promotes storage effects (Sandgren 1983; Angert *et al.* 2009; Li & Chesson 2016). In the experiment, the encystment of *D. cylindricum* was paradigmatic. The proportion of encysting cells increased coherently to nutrient additions – either in the P enrichment or in the N imbalance gradient. When the populations were larger, with intraspecific competition likely higher, the encystment rate increased, promoting a storage effect. The number of species that the methods used allowed observing may still be a small part of the total richness in the lake if we consider the total number of protist and prokaryote individuals present in the lake. We may wonder how many species have stable long-term populations that we never observe.

5.2 Conclusions

Bacterioplankton seasonality in ice-covered lakes

- Three types of OTUs seasonal patterns of the Bacteria assemblages have been identified: resistant to any condition, resilient to harmful high-radiation and opportunistically growing in harsh epilimnetic conditions.
- The great depth of the lake appears as a key factor for the resilience of the core community as it provides refuge against high radiation and temperatures.
- Ecological thresholds related to ice cover duration and the lake size may be relevant factors characterizing bacterioplankton regime shifts in high mountain lakes.

Protist coexistence in oligotrophic lakes

- There is high taxonomic rank clustering across P gradients but niche differentiation at low taxonomic ranks.
- N plays a niche stabilizing role by different sensitivity to N imbalance and resource partitioning concerning the form of N supply.
- Maximum growth rates among protist are frequency-dependent, which may be indicative of intraspecific competition slowing down growth.
- The planktonic protist assemblage is a complex network of interactions where autotrophs and heterotrophs organisms react similarly to limiting and niche differentiating nutrients.
- The negative correlation between proxies of relative fitness and niche differentiation indicates a likely long-term coexistence of a rich protist community.

Bacterioplankton response to nutrient enrichments

- P enrichments have direct and indirect effects over bacteria, being the latter more common and mediated by productive phytoplankton peaks.
- There is little high taxonomic rank differentiation in the responses to nutrient enrichments; however, OTUs from the same families segregate across the P gradient and the form of N supply. This is an indication of niche differentiation between bacterioplankton species that otherwise can show preserved similar ecological features related to its common high taxonomic rank evolutionary history.

The hidden (stable) microbial plankton community

- Microbial plankton species have a great capacity to persist in the water column at low densities and recover high growth rates under favourable conditions.

- Coexistence by storage effects can result from these remaining low-density populations and can be enhanced by resting stages in the sediments.
- The existence of a rare biosphere concerning prokaryotic organisms was part of the contemporary paradigm of planktonic microbial communities. The results of the ENEX experiment indicate that the diversity of the local protist community may be larger than commonly suspected.

APPENDIX

Appendix 1: Taxonomic adscription of the OTU distinguished. x in ENEX column refers to the 122 OTUs of the experiment

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_1	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	NA	x
OTU_10	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	NA	NA	
OTU_100	Unclassified	NA	NA	NA	NA	NA	NA	x
OTU_1005	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	
OTU_1015	Bacteria	Elusimicrobia	Endomicrobia	NA	NA	NA	NA	
OTU_1019	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	NA	
OTU_102	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	NA	
OTU_1024	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	NA	
OTU_103	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	NA	x
OTU_104	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_1041	Bacteria	OD1	Mb-NB09	NA	NA	NA	NA	
OTU_1048	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_105	Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	NA	
OTU_1056	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA	NA	
OTU_106	Bacteria	Chloroflexi	Chloroflexi	[Roseiflexales]	NA	NA	NA	x
OTU_107	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_1075	Bacteria	TM7	SC3	NA	NA	NA	NA	
OTU_108	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_1085	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_1088	Bacteria	TM7	TM7-3	NA	NA	NA	NA	
OTU_109	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	
OTU_11	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	x
OTU_110	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Mycobacteriaceae	Mycobacterium	NA	x
OTU_1108	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_1109	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_111	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_112	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_113	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Prosthecobacter	NA	
OTU_1134	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	NA	NA	
OTU_1137	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	NA	NA	NA	
OTU_114	Bacteria	Verrucomicrobia	[Methylacidiphilae]	Methylacidiphilales	LD19	NA	NA	x
OTU_115	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	NA	
OTU_116	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	NA	NA	
OTU_117	Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	Candidatus Solibacter	NA	
OTU_119	Bacteria	Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae	NA	NA	
OTU_120	Unclassified	NA	NA	NA	NA	NA	NA	x
OTU_1204	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_1219	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Psychrobacter	celer	
OTU_122	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_1221	Archaea	Euryarchaeota	Methanomicrobia	Methanomicrobiales	Methanoregulaceae	Candidatus Methanoregula	NA	
OTU_1222	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	NA	
OTU_1227	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_1229	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_123	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	NA	NA	x
OTU_124	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Zymomonas	NA	x
OTU_1247	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_1249	Bacteria	OD1	SM2F11	NA	NA	NA	NA	
OTU_125	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	NA	x
OTU_126	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	x

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_127	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_128	Bacteria	Acidobacteria	Solibacteres	Solibacterales	Solibacteraceae	Candidatus Solibacter	NA	
OTU_1286	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	NA	
OTU_129	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_1291	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_13	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	x
OTU_130	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	NA	x
OTU_1304	Bacteria	Firmicutes	Clostridia	Clostridiales	Clostridiaceae	Clostridium	perfringens	
OTU_1305	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_1307	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter	NA	
OTU_1308	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	NA	
OTU_131	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	NA	
OTU_132	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_133	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_134	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_135	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_1355	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_136	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	NA	NA	
OTU_137	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_138	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_14	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_140	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_141	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_1415	Bacteria	Acidobacteria	[Chloracidobacteria]	RB41	Ellin6075	NA	NA	
OTU_142	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	x

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_1428	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Spirosoma	NA	
OTU_143	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	NA	NA	
OTU_144	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_145	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	x
OTU_1452	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	NA	
OTU_1455	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_146	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_1461	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA	NA	
OTU_149	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_1496	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bosea	genosp.	
OTU_1497	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Kocuria	NA	
OTU_1498	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	NA	NA	
OTU_15	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_150	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	NA	
OTU_1506	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_151	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_152	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_1521	Bacteria	Proteobacteria	Alphaproteobacteria	NA	NA	NA	NA	
OTU_1531	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	Crenotrichaceae	Crenothrix	NA	
OTU_1534	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_154	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_1548	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_155	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	x
OTU_156	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	NA	x
OTU_157	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_158	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_159	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	NA	NA	NA	
OTU_16	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	NA	x
OTU_160	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	NA	
OTU_162	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	NA	NA	x
OTU_163	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Polynucleobacter	NA	x
OTU_164	Bacteria	Armatimonadetes	[Fimbriimonadia]	[Fimbriimonadales]	[Fimbriimonadaceae]	Fimbriimonas	NA	
OTU_1648	Bacteria	Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Anaerococcus	NA	
OTU_165	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_1656	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_1663	Bacteria	Firmicutes	Bacilli	Gemellales	Gemellaceae	NA	NA	
OTU_1673	Bacteria	Proteobacteria	Gammaproteobacteria	Pasteurellales	Pasteurellaceae	Haemophilus	parainfluenzae	
OTU_1674	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_168	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_1681	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	NA	
OTU_1691	Bacteria	Proteobacteria	Deltaproteobacteria	Spirobacillales	NA	NA	NA	
OTU_1698	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	NA	NA	
OTU_17	Bacteria	Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	NA	NA	
OTU_170	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	NA	
OTU_1705	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Campylobacteraceae	Sulfurospirillum	NA	
OTU_1707	Archaea	Euryarchaeota	Methanomicrobia	Methanosarcinales	Methanosaetaceae	Methanosaeta	NA	
OTU_171	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	Microthrixaceae	NA	NA	
OTU_1718	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA	NA	
OTU_172	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_1720	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	u114	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_1729	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_173	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_1733	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_174	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	NA	x
OTU_175	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	NA	
OTU_1753	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	
OTU_176	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_1764	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_1767	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	NA	
OTU_177	Bacteria	Actinobacteria	Actinobacteria	NA	NA	NA	NA	
OTU_1775	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_178	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA	NA	
OTU_1789	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_1799	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NA	NA	
OTU_18	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_180	Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales	NA	NA	NA	
OTU_181	Bacteria	Proteobacteria	Gammaproteobacteria	Alteromonadales	Alteromonadaceae	NA	NA	
OTU_1816	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_182	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_183	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	NA	
OTU_184	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	NA	NA	x
OTU_185	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_187	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans	NA	
OTU_188	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	NA	
OTU_1880	Unclassified	NA	NA	NA	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_1888	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_189	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	x
OTU_19	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans	NA	x
OTU_190	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_191	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA	NA	
OTU_1915	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	NA	NA	
OTU_192	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	NA	NA	
OTU_1920	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	Prevotellaceae	Prevotella	NA	
OTU_193	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	NA	
OTU_194	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_195	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Polynucleobacter	NA	x
OTU_196	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	NA	
OTU_197	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_1987	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	NA	NA	
OTU_2	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	x
OTU_20	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans	NA	x
OTU_200	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	NA	NA	
OTU_2007	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_201	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	NA	
OTU_202	Unclassified	NA	NA	NA	NA	NA	NA	x
OTU_203	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	NA	NA	
OTU_204	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	NA	NA	NA	
OTU_205	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	x
OTU_2066	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	NA	
OTU_207	Unclassified	NA	NA	NA	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_2076	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Anoxybacillus	kestanbolensis	
OTU_208	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_2085	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae	NA	NA	
OTU_2086	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax	paradoxus	
OTU_2093	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Rothia	mucilaginosa	
OTU_21	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_210	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	NA	NA	
OTU_211	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	NA	NA	
OTU_2110	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	NA	
OTU_212	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_2125	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	NA	
OTU_213	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter	NA	
OTU_214	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_215	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	NA	NA	NA	
OTU_216	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	NA	
OTU_2178	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_218	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	Achromobacter	NA	x
OTU_219	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA	NA	
OTU_22	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	x
OTU_220	Bacteria	Proteobacteria	Alphaproteobacteria	Ellin329	NA	NA	NA	
OTU_221	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_222	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_223	Bacteria	Spirochaetes	Spirochaetes	Spirochaetales	Spirochaetaceae	Spirochaeta	aurantia	
OTU_224	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_225	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_226	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	NA	NA	
OTU_228	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_229	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_23	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	NA	NA	x
OTU_230	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_231	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_232	Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	NA	
OTU_233	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_234	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	NA	
OTU_235	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_236	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	NA	NA	NA	
OTU_237	Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	NA	
OTU_238	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	NA	
OTU_239	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_24	Bacteria	Verrucomicrobia	Opitutae	[Cerasicoccales]	[Cerasicoccaceae]	NA	NA	x
OTU_240	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_241	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA	NA	
OTU_242	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_243	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Janthinobacterium	lividum	x
OTU_244	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Haliangiaceae	NA	NA	
OTU_245	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Pelagibacteraceae	NA	NA	x
OTU_247	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	NA	x
OTU_248	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	NA	NA	x
OTU_249	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	NA	
OTU_25	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	NA	NA	x

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_250	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_251	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	x
OTU_252	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_253	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_254	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_255	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	NA	NA	
OTU_256	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	NA	x
OTU_257	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_258	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_26	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	x
OTU_260	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_261	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	
OTU_262	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_265	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	NA	
OTU_266	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_267	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	NA	NA	
OTU_268	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_269	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	NA	NA	
OTU_27	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Ca. Xiphinematobacter	NA	
OTU_270	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_271	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_273	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	NA	
OTU_274	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	NA	
OTU_275	Unclassified	NA	NA	NA	NA	NA	NA	x
OTU_276	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	x

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_277	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	
OTU_278	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	NA	
OTU_279	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA	NA	
OTU_280	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	NA	
OTU_281	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA	NA	x
OTU_282	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	
OTU_283	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_284	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	NA	
OTU_285	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	NA	NA	NA	x
OTU_286	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_288	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_289	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	
OTU_29	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	NA	NA	x
OTU_290	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	NA	NA	NA	
OTU_291	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	NA	
OTU_292	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	
OTU_293	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_294	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_295	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_297	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	NA	
OTU_299	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NA	NA	x
OTU_3	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	NA	NA	
OTU_30	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	x
OTU_300	Bacteria	TM7	SC3	NA	NA	NA	NA	
OTU_301	Bacteria	Proteobacteria	Deltaproteobacteria	MIZ46	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_302	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_303	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	
OTU_304	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_305	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	NA	NA	
OTU_306	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	OM27	NA	NA	
OTU_307	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_308	Bacteria	Planctomycetes	Planctomycetia	Gemmatales	Gemmataceae	Gemmata	NA	
OTU_309	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter	NA	
OTU_31	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	x
OTU_310	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_311	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_312	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_313	Bacteria	Proteobacteria	Deltaproteobacteria	Spirobacillales	NA	NA	NA	
OTU_315	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	
OTU_316	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Perlucidibaca	NA	
OTU_317	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	NA	
OTU_319	Bacteria	Planctomycetes	Planctomycetia	Gemmatales	Isosphaeraceae	NA	NA	
OTU_32	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Alcaligenaceae	NA	NA	x
OTU_320	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_321	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	Legionella	NA	
OTU_322	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_323	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	NA	NA	NA	
OTU_324	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	NA	
OTU_325	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_326	Bacteria	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_330	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	stutzeri	x
OTU_332	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	NA	x
OTU_333	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_334	Bacteria	Acidobacteria	[Chloracidobacteria]	RB41	Ellin6075	NA	NA	
OTU_335	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_338	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	x
OTU_339	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	NA	NA	
OTU_34	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	x
OTU_340	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_341	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_342	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	
OTU_343	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	NA	
OTU_345	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_346	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Herminiimonas	NA	x
OTU_347	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_35	Bacteria	Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	R4-41B	NA	NA	
OTU_350	Bacteria	TM7	TM7-3	NA	NA	NA	NA	
OTU_351	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	Gaiellaceae	NA	NA	
OTU_352	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	
OTU_353	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	NA	
OTU_354	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_355	Bacteria	TM6	SJA-4	S1198	NA	NA	NA	
OTU_357	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_358	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	Crenotrichaceae	Crenothrix	NA	
OTU_36	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_360	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	NA	
OTU_361	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_362	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	NA	NA	NA	
OTU_363	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_364	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	NA	
OTU_365	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_366	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_367	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Conexibacteraceae	NA	NA	
OTU_369	Bacteria	Firmicutes	Bacilli	Lactobacillales	Enterococcaceae	Enterococcus	cecorum	
OTU_37	Bacteria	TM7	TM7-1	NA	NA	NA	NA	
OTU_370	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_371	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_372	Bacteria	Chloroflexi	SL56	NA	NA	NA	NA	
OTU_373	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_374	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Polyangiaceae	NA	NA	
OTU_375	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	NA	
OTU_377	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	NA	NA	
OTU_378	Bacteria	Proteobacteria	Gammaproteobacteria	Enterobacteriales	Enterobacteriaceae	NA	NA	
OTU_379	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA	NA	
OTU_38	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	NA	NA	
OTU_382	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	NA	
OTU_383	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_384	Bacteria	Proteobacteria	Betaproteobacteria	SBla14	NA	NA	NA	
OTU_385	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_386	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_387	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Herminiimonas	NA	
OTU_389	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	
OTU_39	Unclassified	NA	NA	NA	NA	NA	NA	x
OTU_390	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	Crenotrichaceae	Crenothrix	NA	
OTU_391	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	NA	
OTU_392	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_393	Bacteria	Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Finegoldia	NA	
OTU_394	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_395	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_396	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	NA	x
OTU_397	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_398	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	NA	NA	NA	x
OTU_399	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Curvibacter	NA	
OTU_4	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_40	Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	NA	NA	
OTU_401	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_402	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Sulfuricurvum	kujiense	
OTU_403	Bacteria	Armatimonadetes	Armatimonadia	Armatimonadales	Armatimonadaceae	NA	NA	
OTU_404	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	
OTU_405	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_406	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_407	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_408	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	NA	
OTU_409	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Herminiimonas	NA	
OTU_41	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_410	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	yabuuchiae	x
OTU_411	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA	NA	
OTU_412	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_414	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_415	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_417	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	NA	
OTU_418	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Ralstonia	NA	x
OTU_419	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	NA	x
OTU_42	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA	NA	
OTU_420	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_421	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_424	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_425	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Methylibium	NA	
OTU_426	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_427	Bacteria	TM7	SC3	NA	NA	NA	NA	
OTU_428	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_429	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_43	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA	NA	
OTU_430	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Micrococcus	luteus	
OTU_431	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	NA	NA	
OTU_433	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	NA	NA	
OTU_435	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_436	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_437	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Novosphingobium	NA	
OTU_438	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_439	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingobium	NA	x
OTU_44	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_441	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	NA	NA	
OTU_442	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Sinobacteraceae	Nevskia	NA	
OTU_446	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_447	Bacteria	Proteobacteria	Deltaproteobacteria	MIZ46	NA	NA	NA	
OTU_448	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_449	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_45	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_450	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	x
OTU_451	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_452	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_453	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Enhydrobacter	NA	
OTU_454	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_455	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_457	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	NA	
OTU_458	Bacteria	Verrucomicrobia	Opitutae	[Cerasicoccales]	[Cerasicoccaceae]	NA	NA	
OTU_459	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_46	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	x
OTU_461	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_462	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_464	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	NA	
OTU_466	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Flectobacillus	NA	x
OTU_467	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_469	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_47	Unclassified	NA	NA	NA	NA	NA	NA	x
OTU_47	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_470	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_471	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_473	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA	NA	
OTU_474	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_475	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	
OTU_476	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	NA	x
OTU_477	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_479	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	NA	NA	
OTU_48	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Chthoniobacter	NA	
OTU_480	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_481	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	Sulfurimonas	NA	
OTU_483	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_484	Bacteria	TM7	TM7-1	NA	NA	NA	NA	
OTU_485	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Collimonas	NA	
OTU_486	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Mycoplana	NA	
OTU_487	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	Patulibacteraceae	Patulibacter	NA	
OTU_488	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	NA	NA	
OTU_489	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	Crenotrichaceae	Crenothrix	NA	
OTU_490	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_491	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	NA	NA	
OTU_492	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_493	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_495	Unclassified	NA	NA	NA	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_497	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_498	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	NA	NA	
OTU_5	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Polaromonas	NA	x
OTU_50	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	Crenotrichaceae	Crenothrix	NA	
OTU_500	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_501	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_502	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_504	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_505	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_508	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Leptothrix	NA	
OTU_509	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_51	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	x
OTU_510	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	Crenotrichaceae	Crenothrix	NA	
OTU_511	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_512	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Nocardiaceae	Rhodococcus	fascians	
OTU_513	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_514	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	NA	
OTU_519	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	NA	NA	
OTU_52	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	NA	NA	
OTU_520	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax	paradoxus	
OTU_523	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	
OTU_525	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Rhodoplanes	NA	
OTU_526	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_528	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_53	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_533	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	
OTU_534	Bacteria	Proteobacteria	Gammaproteobacteria	NA	NA	NA	NA	
OTU_535	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Phenylobacterium	NA	
OTU_537	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_539	Bacteria	Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	WAL_1855D	NA	
OTU_54	Bacteria	Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	auto67_4W	NA	NA	
OTU_540	Bacteria	Proteobacteria	Alphaproteobacteria	NA	NA	NA	NA	
OTU_541	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_546	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_55	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	succinicans	x
OTU_550	Bacteria	Actinobacteria	Thermoleophilia	Solirubrobacterales	NA	NA	NA	
OTU_551	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	NA	
OTU_552	Bacteria	Acidobacteria	Holophagae	Holophagales	Holophagaceae	Geothrix	NA	
OTU_553	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	Sphingobacteriaceae	NA	NA	
OTU_555	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	NA	NA	
OTU_556	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_557	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	
OTU_558	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	
OTU_559	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Kaistobacter	NA	
OTU_56	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	NA	NA	NA	
OTU_560	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	NA	NA	NA	
OTU_562	Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	NA	NA	
OTU_563	Bacteria	Proteobacteria	Deltaproteobacteria	Myxococcales	Myxococcaceae	Anaeromyxobacter	NA	
OTU_564	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	
OTU_565	Unclassified	NA	NA	NA	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_566	Bacteria	Proteobacteria	Deltaproteobacteria	MIZ46	NA	NA	NA	
OTU_567	Bacteria	Proteobacteria	Epsilonproteobacteria	Campylobacterales	Helicobacteraceae	NA	NA	
OTU_569	Bacteria	TM7	TM7-3	NA	NA	NA	NA	
OTU_57	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	x
OTU_570	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Hymenobacter	NA	
OTU_571	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Herminiimonas	NA	x
OTU_572	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae	Methylobacterium	adhaesivum	
OTU_573	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	NA	NA	
OTU_574	Bacteria	Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	NA	NA	
OTU_578	Bacteria	Acidobacteria	Holophagae	Holophagales	Holophagaceae	Geothrix	NA	
OTU_579	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	NA	
OTU_58	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	x
OTU_580	Bacteria	WS5	NA	NA	NA	NA	NA	
OTU_581	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_582	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Acetobacteraceae	NA	NA	
OTU_584	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_585	Bacteria	Firmicutes	Bacilli	Lactobacillales	Streptococcaceae	Streptococcus	NA	
OTU_586	Bacteria	Firmicutes	Bacilli	Bacillales	Staphylococcaceae	Staphylococcus	NA	
OTU_587	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_588	Bacteria	Nitrospirae	Nitrospira	Nitrospirales	Nitrospiraceae	Nitrospira	NA	
OTU_589	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	NA	
OTU_59	Bacteria	Planctomycetes	Phycisphaerae	Phycisphaerales	NA	NA	NA	x
OTU_590	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	NA	NA	
OTU_591	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	NA	
OTU_593	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Salinibacterium	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_596	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Saprospiraceae	Haliscomenobacter	NA	
OTU_597	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_598	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	NA	
OTU_599	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	
OTU_6	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_60	Bacteria	Proteobacteria	Alphaproteobacteria	Ellin329	NA	NA	NA	
OTU_600	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	NA	x
OTU_601	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_603	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_604	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Hyphomicrobiaceae	Devosia	NA	
OTU_607	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	
OTU_608	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	NA	
OTU_609	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_61	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	NA	NA	
OTU_611	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bdellovibrionaceae	Bdellovibrio	NA	
OTU_612	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	Acinetobacter	lwoffii	
OTU_614	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Cytophaga	NA	
OTU_615	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_616	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	NA	NA	
OTU_617	Bacteria	Proteobacteria	Deltaproteobacteria	Bdellovibrionales	Bacteriovoracaceae	NA	NA	
OTU_618	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter	NA	
OTU_62	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	x
OTU_620	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_623	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_625	Unclassified	NA	NA	NA	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_629	Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	NA	NA	
OTU_63	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans	NA	x
OTU_635	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_639	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_64	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	x
OTU_643	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_644	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_645	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	NA	NA	
OTU_646	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	fragi	
OTU_65	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_650	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_651	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_653	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	Clavibacter	NA	
OTU_654	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_656	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_66	Bacteria	Bacteroidetes	Cytophagia	Cytophagales	Cytophagaceae	Flectobacillus	NA	x
OTU_663	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_666	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_669	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_67	Bacteria	Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	R4-41B	NA	NA	
OTU_675	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_677	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	NA	NA	
OTU_678	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_68	Bacteria	Verrucomicrobia	Opitutae	Opitutales	Opitutaceae	Opitutus	NA	
OTU_680	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_681	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter	NA	
OTU_682	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_684	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_685	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	NA	NA	
OTU_686	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfobacterales	Desulfobacteraceae	NA	NA	
OTU_687	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_688	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_689	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_69	Bacteria	Verrucomicrobia	Opitutae	[Cerasicoccales]	[Cerasicoccaceae]	NA	NA	
OTU_690	Bacteria	Bacteroidetes	Bacteroidia	Bacteroidales	NA	NA	NA	
OTU_692	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	Bosea	genosp.	
OTU_693	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_696	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_698	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_7	Bacteria	Verrucomicrobia	Opitutae	[Cerasicoccales]	[Cerasicoccaceae]	NA	NA	
OTU_70	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_700	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_702	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Coxiellaceae	NA	NA	
OTU_703	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_705	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_706	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_708	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	NA	NA	
OTU_71	Bacteria	Actinobacteria	Thermoleophilia	Gaiellales	NA	NA	NA	
OTU_713	Bacteria	Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae	Sulfuritalea	NA	
OTU_714	Bacteria	Proteobacteria	Betaproteobacteria	Methylophilales	Methylophilaceae	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_716	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	Herminiimonas	NA	
OTU_719	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_72	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	
OTU_723	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	NA	
OTU_724	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Cryomorphaceae	Fluviicola	NA	
OTU_725	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_729	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	diminuta	
OTU_73	Bacteria	Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae	Sphingomonas	echinoides	x
OTU_735	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	Ca. Xiphinematobacter	NA	
OTU_736	Bacteria	Verrucomicrobia	[Pedosphaerae]	[Pedosphaerales]	auto67_4W	NA	NA	
OTU_74	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	x
OTU_741	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Bradyrhizobiaceae	NA	NA	
OTU_742	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Holosporaceae	NA	NA	
OTU_743	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_744	Bacteria	Planctomycetes	Planctomycetia	Planctomycetales	Planctomycetaceae	Planctomyces	NA	
OTU_745	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	u114	NA	
OTU_747	Bacteria	Proteobacteria	Gammaproteobacteria	Methylococcales	Crenotrichaceae	Crenothrix	NA	
OTU_748	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_749	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Variovorax	paradoxus	
OTU_75	Bacteria	Bacteroidetes	Sphingobacteriia	Sphingobacteriales	NA	NA	NA	
OTU_750	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_751	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_76	Bacteria	Gemmatimonadetes	Gemmatimonadetes	KD8-87	NA	NA	NA	
OTU_767	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_769	Unclassified	NA	NA	NA	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_77	Bacteria	Actinobacteria	Acidimicrobiia	Acidimicrobiales	C111	NA	NA	
OTU_770	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Kocuria	palustris	
OTU_771	Bacteria	Proteobacteria	Deltaproteobacteria	Desulfuromonadales	Geobacteraceae	Geobacter	NA	
OTU_775	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_778	Bacteria	Proteobacteria	Alphaproteobacteria	Rhizobiales	Beijerinckiaceae	NA	NA	
OTU_78	Bacteria	Acidobacteria	Solibacteres	Solibacterales	NA	NA	NA	
OTU_781	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	Pseudomonas	NA	
OTU_782	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_785	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_786	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_79	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	x
OTU_798	Bacteria	Firmicutes	Bacilli	NA	NA	NA	NA	
OTU_8	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	x
OTU_80	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	NA	NA	x
OTU_801	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_806	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	NA	NA	
OTU_807	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	aminovorans	
OTU_808	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_809	Bacteria	Actinobacteria	Actinobacteria	Bifidobacteriales	Bifidobacteriaceae	Bifidobacterium	thermacidophilum	
OTU_810	Bacteria	Firmicutes	Bacilli	Bacillales	Planococcaceae	Planomicrobium	NA	
OTU_811	Bacteria	Firmicutes	Bacilli	Bacillales	Bacillaceae	Bacillus	coagulans	
OTU_812	Bacteria	Bacteroidetes	Flavobacteriia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	NA	
OTU_814	Bacteria	Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Anaerococcus	NA	
OTU_819	Bacteria	Proteobacteria	Betaproteobacteria	Neisseriales	Neisseriaceae	NA	NA	
OTU_82	Bacteria	Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	NA	NA	x

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_820	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Corynebacteriaceae	Corynebacterium	NA	
OTU_822	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	NA	NA	NA	
OTU_827	Bacteria	Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae	NA	NA	
OTU_828	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_829	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	NA	NA	NA	
OTU_83	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	NA	x
OTU_830	Bacteria	Firmicutes	Clostridia	Clostridiales	[Tissierellaceae]	Peptoniphilus	NA	
OTU_831	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Micrococcaceae	Rothia	dentocariosa	
OTU_835	Bacteria	Verrucomicrobia	[Spartobacteria]	[Chthoniobacterales]	[Chthoniobacteraceae]	NA	NA	
OTU_84	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	NA	x
OTU_85	Bacteria	Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae	NA	NA	x
OTU_852	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	NA	
OTU_856	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_858	Bacteria	Fusobacteria	Fusobacteriia	Fusobacteriales	Fusobacteriaceae	u114	NA	
OTU_86	Bacteria	Verrucomicrobia	Verrucomicrobiae	Verrucomicrobiales	Verrucomicrobiaceae	Luteolibacter	NA	
OTU_862	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_869	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Limnohabitans	NA	
OTU_87	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_870	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_88	Bacteria	Proteobacteria	Betaproteobacteria	NA	NA	NA	NA	
OTU_880	Unclassified	NA	NA	NA	NA	NA	NA	
OTU_89	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Leptothrix	NA	x
OTU_890	Bacteria	Proteobacteria	Alphaproteobacteria	Rhodospirillales	Rhodospirillaceae	NA	NA	
OTU_899	Bacteria	Verrucomicrobia	Opitutae	[Cerasicoccales]	[Cerasicoccaceae]	NA	NA	
OTU_9	Bacteria	Actinobacteria	NA	NA	NA	NA	NA	

OTU	Kingdom	Phylum	Class	Order	Family	Genus	Species	ENEX
OTU_90	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	NA	NA	x
OTU_905	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	
OTU_91	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	x
OTU_92	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_920	Bacteria	Proteobacteria	Alphaproteobacteria	Rickettsiales	Rickettsiaceae	Rickettsia	NA	
OTU_93	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae	Rhodoferax	NA	x
OTU_940	Bacteria	Proteobacteria	Betaproteobacteria	Burkholderiales	NA	NA	NA	
OTU_95	Bacteria	Planctomycetes	Planctomycetia	Pirellulales	Pirellulaceae	NA	NA	
OTU_950	Bacteria	Bacteroidetes	[Saprospirae]	[Saprospirales]	Chitinophagaceae	Sediminibacterium	NA	
OTU_96	Bacteria	Gemmatimonadetes	Gemmatimonadetes	KD8-87	NA	NA	NA	
OTU_97	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	x
OTU_98	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae	NA	NA	
OTU_987	Bacteria	Actinobacteria	Actinobacteria	Actinomycetales	ACK-M1	NA	NA	
OTU_99	Unclassified	NA	NA	NA	NA	NA	NA	

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