ORIGIN AND FATE OF ODOROUS METABOLITES, 2-METHYLISOBORNEOL AND GEOSMIN, IN A EUTROPHIC RESERVOIR

Nicolas André Clercin

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Doctoral Committee

_____

Gregory K. Druschel, PhD, Chair

_____

Pierre-André Jacinthe, PhD

November 13, 2018

_____

Gabriel Filippelli, PhD

_____

Max Jacobo Moreno-Madriñán, PhD

_____

Sarath Chandra Janga, PhD

DEDICATION

I would like to dedicate this work to my family, my wife Angélique and our three sons Pierre-Adrien, Aurélien and Marceau. I am aware that the writing of this manuscript has been an intrusion into our daily life and its achievement now closes the decade-long 'Indiana' chapter of our family.

Another dedication to my parents and my young brother who have always been supportive and respectful of my choices even if they never fully understood the content of my research. A special thought to my dad (†2005) who loved so much sciences and technologies but never got the chance to study as a kid. Him who idolized his own father, a WWII resistant but became head of the family upon his father's death when he was only 8. Him who had to work to support his widowed mother and his two younger brothers. Him who decided to join the French navy at the age of 16 as a seaman recruit in order to finally reach his personal goal and study, learn diesel engine mechanics, a skill that served him later in the civilian life. You are my model of perseverance and I know this new milestone in my life is your pride.

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Nicolas André Clercin

ORIGIN AND FATE OF ODOROUS METABOLITES,
2-METHYLISOBORNEOL AND GEOSMIN,
IN A EUTROPHIC RESERVOIR

Taste-and-Odor (T&O) occurrences are a worldwide problem and can locally have extensive socio-economic impacts in contaminated waterbodies. Tracing odorous compounds in surface waters or controlling the growth of producing organisms is particularly challenging. These approaches require the understanding of complex interactions between broad climate heterogeneity, large-scale physical processes such basin hydrology, lake/reservoir circulation, responses of aquatic ecosystems and communities. Eagle Creek Reservoir (ECR), a eutrophic water body, located in central Indiana experiences annual odorous outbreaks of variable durations and intensities that can impair its water quality. Two major compounds, 2-methylisoborneol and geosmin, have been identified as the main culprits occurring seasonally when the reservoir receives high discharges and nutrient loads from its main tributaries. Under these conditions, the growth of T&O-producing bacteria tends to take over other phytoplanktic organisms. Discrete samples collected within the water column during severe outbreaks in 2013 revealed that some bacterioplankton members belonging to Actinobacteria (*Streptomyces*) and Cyanobacteria (*Planktothrix*) were involved in the generation of T&O compounds. Most of this production occurred in the upper layers of the water column where higher abundances of key enzymes from MIB and geosmin metabolic pathways were detected. Application of a copper-based algaecide to curb the biosynthesis of bacterial metabolites led to geosmin production (linked to Cyanobacteria) being quickly terminated, whereas MIB levels (linked to Actinobacteria) lingered for several weeks after the algaecide treatment.

Significant chemical differences in the association of these metabolites were measured in ECR. Geosmin was dominantly found cell-bound and settling after cellular death increases susceptibility to biodegradation in bottom sediments. MIB was mostly found dissolved making it less susceptible to biodegradation in bottom sediments. Genetic data identified *Novosphingobium hassiacum* and *Sphingomonas oligophenolica* (α-

Proteobacteria) as potential degraders of geosmin and, four *Flavobacterium* species (Bacteroidetes) as potential MIB degraders. The role of Eagle Creek natural sediments in the removal of bacterial metabolites via chemical adsorption was also tested but was not proven efficient. Bacterial breakdown activity was demonstrated to be the major loss mechanism of MIB and geosmin.

Gregory K. Druschel, PhD, Chair

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LIST OF ABBREVIATIONS

CCA:	Canonical Correspondence Analysis
DOM:	Dissolved Organic Matter
EC:	Enzyme Commission number
ECR:	Eagle Creek Reservoir
FPP:	Farnesyl Pyrophosphate
GAC:	Granular Activated Carbon
GPP:	Geranyl Pyrophosphate
GSM:	Geosmin
LOD:	Limit of Detection
MCL:	Maximum Concentration Level
MCLG:	Maximum Concentration Level Goal
MEP:	2-methyl-erythritol-4-phosphate
MIB:	2-methyl-isoborneol
MVA:	Mevalonate
NOM:	Natural Organic Matter
OTC:	Odor Threshold Concentration
OTU:	Operational Taxonomic Unit
PAC:	Powdered Activated Carbon
RTRM:	Relative Thermal Resistance to Mixing
T&O:	Taste-and-Odor

LIST OF ACRONYMS

IUPUI:	Indiana University – Purdue University, Indianapolis
USEPA:	U.S. Environmental Protection Agency
USGS:	U.S. Geological Survey

CHAPTER 1 – INTRODUCTION

Brief history

Eagle Creek Reservoir, one of the three water supply reservoirs located in the Upper White River watershed, provides drinking water to the City of Indianapolis. Since strong T&O events that occurred in the early 2000's, central Indiana drinking water supply reservoirs; namely Geist, Morse and Eagle Creek reservoirs, have been frequently impacted by redundant and sometimes severe taste-and-odor (T&O) issues which deteriorate the quality of source waters. To identify the source of the problem, the local water company began to investigate the origin of these T&O events. Seasonally, finished water produced in drinking water treatment plants was tainted by odorous volatile compounds. Primary investigations identified two organic compounds, 2-methylisoborneol and geosmin, as the main culprits. Next, the monitoring of these odorous compounds within water treatment plants, from the intake to the tap, rapidly extended to streams and reservoirs where raw water was taken from. In the case of Eagle Creek, a long-term research and development partnership between the water company and the Center for Earth and Environmental Science at IUPUI emerged. In 2004, the new Central Indiana Water Resources Partnership joined its forces with the Eagle Creek Watershed Task Force to create the Eagle Creek Watershed Alliance, a broad coalition of citizens, volunteers, county, state and federal agencies, universities and water managers who can work together for the improvement of the Eagle Creek water quality, raising public awareness and encouraging the stewardship of the watershed's resources [*Tedesco et al.*, 2005].

Taste-and-Odor Issues

Episodes of taste-and-odor nuisance of water bodies have been reported worldwide [*Juttner and Watson*, 2007; *Krishnani et al.*, 2008]. The production of odorous compounds by aquatic organisms is a significant concern for water utilities and the majority of T&O outbreaks in drinking water supplies are caused by microbial growth [*Juttner and Watson*, 2007]. In natural environments, unpleasant T&O occurrences of the two smelling

terpenoids, geosmin (GSM; *trans*-1,10-dimethyl-*trans*-9-decalol) and 2-methylisoborneol (MIB) are frequently reported [*Lanciotti et al.*, 2003; *Ma et al.*, 2013; *Watson et al.*, 2008; *Westerhoff et al.*, 2005]. Geosmin and MIB are the main metabolites causing T&O problems in drinking water [*Watson et al.*, 2008]. They impart an earthy (GSM) and musty (MIB) taint to the water [*Izaguirre and Taylor*, 2004b] and/or to fish flesh in aquaculture [*Klausen et al.*, 2005; *Robin et al.*, 2006]. Each compound exists as (+) and (-) enantiomers (Figure 1.1) but T&O outbreaks in nature occur as the (-) stereoisomer which is ten times more potent than its (+) counterpart [*Juttner and Watson*, 2007]. Odor threshold concentrations of geosmin and MIB are very low, thus concentrations at parts per trillion levels (or ng L^{-1}) can easily be detected by human olfactory sense [*Peter and Von Gunten*, 2007].

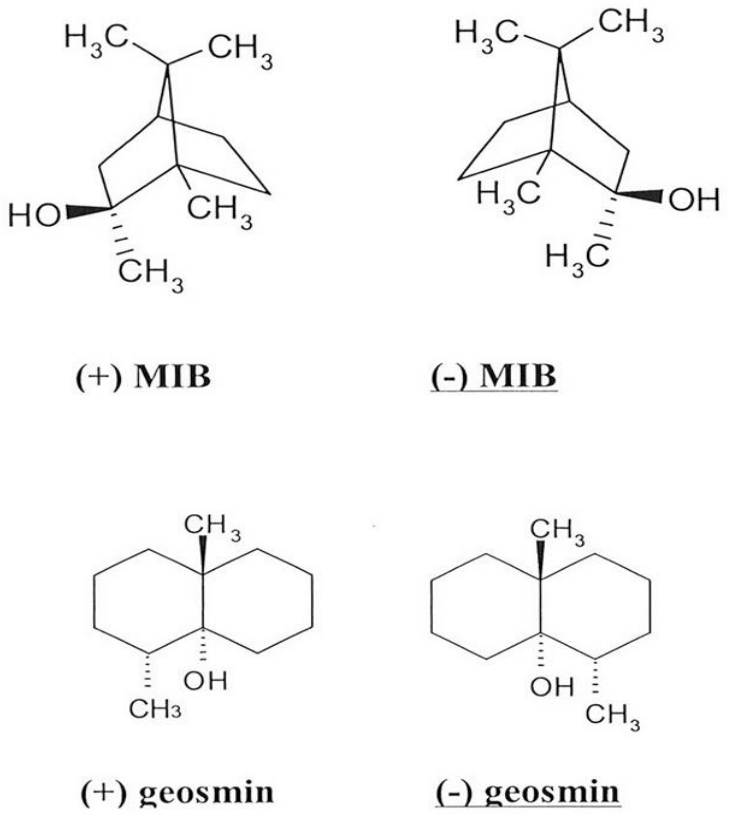

Figure 1.1: Chemical structures of MIB and GSM enantiomers

Known sources of T&O

The most important source of geosmin and MIB are bacteria, which include a broad variety of non-related organisms such as Actinobacteria, Cyanobacteria and Myxobacteria [*Juttner and Watson*, 2007; *Watson*, 2003]. In aquatic environments, major producers of MIB and geosmin are Cyanobacteria [*Juttner and Watson*, 2007; *Watson et al.*, 2008]. Although Actinobacteria are known to be the main producers of odorous metabolites in soil [*Juttner*, 1990; *Zaitlin and Watson*, 2006], these organisms are also very frequently found in lake sediments where they play a significant role in organic matter degradation [*Jiang and Xu*, 1996]. Soil dwelling Myxobacteria, who also have the ability to synthesize odorous metabolites have been detected in surface waters [*Dickschat et al.*, 2005; *Dickschat et al.*, 2007]. Off-flavor compounds, such as MIB and GSM which deteriorate the quality of water, are often associated with seasonal cyanobacterial blooms of *Oscillatoria, Anaebana flos-aquae, Planktothrix* and *Microcystis aeruginosa* [*Hayes and Burch*, 1989; *Li et al.*, 2007; *Su et al.*, 2015] but decaying blooms are also involved in the release of many odorous metabolites [*Ma et al.*, 2013; *Smith et al.*, 2008] and other bioactive compounds [*Smith et al.*, 2008].

Historically, *Bentley and Meganathan* [1981] were the first to identify the origin of the two volatile compounds MIB and geosmin. They characterized MIB as a methylated monoterpene (C_{11}) alcohol and, geosmin as a degraded sesquiterpenoid (C_{12}) alcohol that has lost an isopropyl group (C_3), both derived from the biosynthesis of isoprene units. These compounds have relatively low molecular weight [*Pirbazari et al.*, 1992], moderate solubility, and moderate hydrophobicity [*Song and O'Shea*, 2007] (Table 1.1). The partitioning of MIB and geosmin in different cellular fractions was observed a few years later but neither compound occurred in solution in the cell cytoplasm. Most GSM was found to be bound to thylakoid and cytoplasmic membranes whereas MIB was less closely bound to membrane proteins [*Juttner and Watson*, 2007; *Wu and Jüttner*, 1988]. A potential linkage of MIB to the photosynthetic apparatus was illustrated by *Bafford et al.* [1993] and later linked to the production of lipophilic and phycobilin pigments [*Zimba et al.*, 1999]. Light is the important parameter which changes cell pigment content and appears to affect the terpenoid production. Low light intensities (< 30 µmol photons. m^{-2}.

s^{-1}) enhance the synthesis of both MIB [*Wang et al.*, 2011] and GSM [*Zhang et al.*, 2009]. Many authors disucssed the importance of light in the production of geosmin in relation to chlorophyll content [*Naes et al.*, 1985], to nutrients [*Naes et al.*, 1988; *Saadoun et al.*, 2001], to temperature [*Rashash et al.*, 1995; *Zhang et al.*, 2009] and pH [*Blevins et al.*, 1995].

Table 1.1: Properties of the odorous metabolites MIB and GSM [*Peter and Von Gunten*, 2007; *Pirbazari et al.*, 1992]

	MIB	**GSM**
CAS #	2371-42-8	19700-21-1
Molecular formula	$C_{11}H_{20}O$	$C_{12}H_{22}O$
Odor	musty	earthy
Odor Threshold Concentration (ng L^{-1})	15.0	4.0
Molecular weight (g mol^{-1})	168.3	182.3
log K_{ow}	3.13	3.70
Aqueous solubility (mg L^{-1}; 25°C)	194.5	150.2
Boiling point (°C)	196.7	165.1
Density (g cm^{-3})	0.9288	0.9494
Vapor pressure (Pa)	6.68	5.49
Henry's law constant (Pa m^3 mol^{-1})	5.76	6.66

Biosynthesis of T&O metabolites

Biosynthetically, terpenes are generated by terpene cyclases (= isoprenoid synthase type I) from linear precursors: geranyl pyrophosphate (GPP), the immediate precursor of C_{10} monoterpenes and, farnesyl pyrophosphate (FPP) of cyclic C_{15} sesquiterpenes [*Cane et al.*, 2006]. Addition of labeled 1-deoxy-D-xylulose to culture of *Streptomyces* [*Spiteller et al.*, 2002] and, labeled mevalolactone and leucine in the myxobacteria (*Myxococcus* and

Stigmatella) resulted in the production of labeled geosmin [*Dickschat et al.*, 2005]. Among others, these two studies helped to reveal that different biosynthetic pathways of isoprene were involved (Figure 1.2). The synthesis of labeled geosmin by addition of [5,4-$^{2}H_{2}$]-1-deoxy-D-xylulose demonstrated that the 2-methylerythritol-4-phosphate (MEP) pathway was predominant in *Streptomyces* whereas the mevalonate (MVA) pathway with addition of [4,4,6,6-$^{2}H_{5}$]-mevalolactone was the preferred route in myxobacteria [*Spiteller et al.*, 2002]. *Kuzuyama* [2002] found the gene coding for the MEP pathway in the cyanobacterium *Synechocystis*. For many bacterial groups, the MEP route is the major biosynthetic pathway. Nonetheless, both MEP and MVA can be used by the same organism. *Dickschat et al.* [2005] showed that Myxobacteria prefer using the MVA route, while Archaea exclusively use this latter pathway although no species have been documented as potential MIB and geosmin producers [*Lange et al.*, 2000]. The enzyme germacradienol/geosmin synthase [E.C: 4.1.99.16] was identified in the actinobacterium *Streptomyces coelicolor* [*Jiang and Cane*, 2008]. Similarly, radio-labeled [methyl-^{13}C] methionine and deuterium [$^{2}H_{5}$]-mevalolactone in the myxobacterium *Nannocystis exedens* revealed the pathway leading to the biosynthesis of MIB [*Dickschat et al.*, 2007] and the identification of an unusual bacterial terpene cyclase, the MIB synthase [E.C: 4.2.3.118] [*Wang and Cane*, 2008]. According to *Rosen et al.* [1992], geosmin is produced during the exponential growth phase and a relatively small fraction is excreted to surrounding water; production stops during the stationary phase, and the cell lysis causes the bulk release of cell-bound geosmin into the medium. In *Streptomyces*, the production of the secondary metabolites occurs during the secondary mycelial growth which requires the presence of oxygen and coincides with sporulation [*Dionigi et al.*, 1992]. In a stratified lake, two independent sources of odorous metabolites were observed: a minor source of geosmin in the epilimnion produced by Actinomycetes and a larger source in the anaerobic hypolimnion where producing microorganisms were not identified [*Henatsch and Jüttner*, 1986]. Due to high sulfide and ammonia concentrations at the bottom of the lake, known aerobic producers such as Actinomycetes and Myxobacteria were ruled out by the two authors.

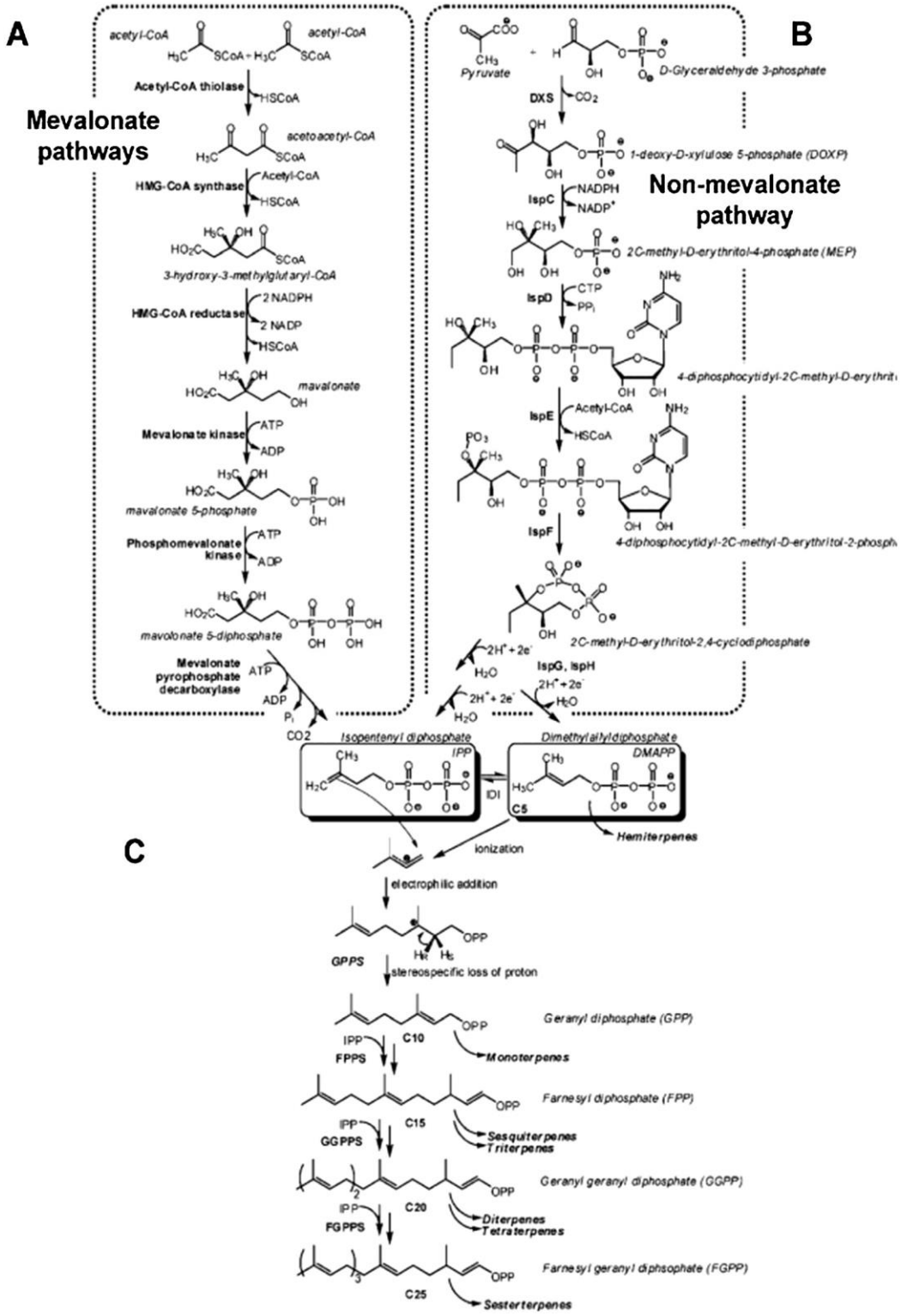

Figure 1.2: Biosynthesis of isoprenoids with A) the mevalonate (MVA); B) the non-mevalonate (MEP) and C) terpene pathways, from *Watson et al.* [2016].

Nuisance of T&O

The major challenge for the water industry is to effectively remove taste-and-odor compounds from the water to levels below the human detection threshold. The Odor Threshold Concentration (OTC) is extremely low for human olfaction and is around 10 ng L^{-1} or less for MIB and GSM [*Ashitani et al.*, 1988; *Rashash et al.*, 1997; *Suffet*, 1987]. While the conventional water treatment process (coagulation/flocculation/sedimentation) is ineffective to get rid of MIB and GSM from drinking water [*Bruce et al.*, 2002], several other physical-chemical treatment processes have been studied: chlorination [*Lalezary et al.*, 1986b], adsorption on granular activated carbon, GAC [*Hrudey et al.*, 1995] or powdered activated carbon, PAC [*Ng et al.*, 2002; *Yuan et al.*, 2013], and ozonation [*Ho et al.*, 2002; *Nerenberg et al.*, 2000]. PAC is an important tool for maintaining the aesthetic quality of drinking water and was proven very useful at adsorbing MIB, geosmin and other contaminants [*Graham et al.*, 2000]. Competitive natural organic matter (NOM) reduces PAC effectiveness, the availability of sorption sites [*Lalezary et al.*, 1986a] and high PAC dosages can be challenging for cost optimization. This reduction of PAC effectiveness can be significant, depending on the nature and concentrations of NOM in water [*Sontheimer et al.*, 1988]. To enhance the removal efficiency of MIB and GSM with PAC in presence of NOM, *Matsui et al.* [2010] suggested the utilization of super-powdered activated carbon (S-PAC) which has finer particles than those of a regular PAC. Unfortunately, the presence in raw water of dissolved organic matter (DOM) usually depletes ozone which favors the incomplete oxidation of MIB and GSM that produces hazardous by-products [*Nerenberg et al.*, 2000]. For this reason, advanced oxidative technologies such as TiO_2 photo-catalysis [*Bellu et al.*, 2008; *Lawton et al.*, 2003], ZnO photo-catalysis [*Sirtori et al.*, 2006] and UV/H_2O_2 [*Rosenfeldt et al.*, 2005] gained credit for a quick and effective removal of MIB and GSM that may go through the filtration step. The degradation of earthy GSM and musty MIB compounds is promoted by hydroxyl radicals [*Jo et al.*, 2011]. Ultrasonic-induced degradation of odorous metabolites at 640 kHz was also proven effective [*Song and O'Shea*, 2007]. Two by-products, 2-methylene-bornane (2-MB) and 2-methyl-2-bornene (2-M-2-B) on Figure 1.3, were identified from the dehydration of MIB [*Manickum and John*, 2012; *Schumann and Pendleton*, 1997] while enone (Figure 1.4) resulted from

degradation of geosmin [*Saito et al.*, 1999]. A new alternative approach, called bio-filtration, consists in using the water treatment plant sand filters as a medium to remove MIB and GSM [*Ho et al.*, 2007; *Hsieh et al.*, 2010; *McDowall et al.*, 2007] which can also be seeded with bacteria able to degrade the odorous compounds [*McDowall et al.*, 2009]. Biodegradation has also shown promise in removal by mixing quartz sand with 10% of lake bed sediments [*DeVries et al.*, 2012].

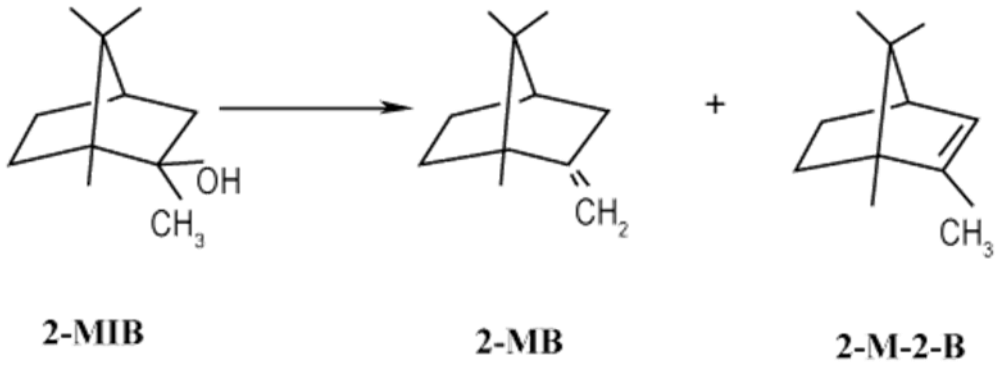

Figure 1.3: Dehydration of 2-methylisoborneol (MIB) into 2-methylenebornane (2-MB) and 2-methyl-2-bornene (2-M-2-B), after *Manickum and John* [2012].

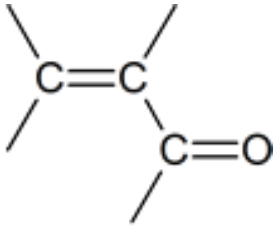

Figure 1.4: General structure of enones

Possible ways of remediation

Interrelationships between tastes and odors in water supplies, the seasonal cycles of freshwater algae (diatoms and cyanobacteria) and the growth of gram-negative bacteria were first hypothesized by *Silvey and Roach* [1964]. Subsequently, a few strains of *Bacillus cereus* were found to effectively degrade geosmin [*Silvey et al.*, 1970]. A few years later, *Bacillus cereus* and *B. subtilis* both isolated from soil-enrichment cultures were readily able to breakdown geosmin [*Narayan and Nunez*, 1974]. A strain of *Pseudomonas putida* that can oxidize MIB was isolated by *Izaguirre et al.* [1988] as well as seven other gram-negative strains of bio-degraders, belonging to the *Pseudomonas* and *Flavobacterium* genera [*Egashira et al.*, 1992]. Since then, many other reports have demonstrated the role of bacteria in the degradation of MIB and geosmin: *Arthrobacter* and *Chlorophenolicus* [*Saadoun and el-Migdadi*, 1998], *Bacillus* [*Ishida and Miyaji*, 1992; *Lauderdale et al.*, 2004], *Pseudomonas* [*Oikawa et al.*, 1995], *Novosphingobium* and *Sphingopyxis* [*Hoefel et al.*, 2006; *Hoefel et al.*, 2009], *Comamonas* and *Variovorax* [*Guttman and van Rijn*, 2012], and *Rhodococcus* [*Eaton and Sandusky*, 2009; 2010; *Guttman and van Rijn*, 2012; *Saadoun and el-Migdadi*, 1998].

Scope of this study

Notwithstanding numerous aquatic organisms being described as potential sources of volatile and odorous compounds in surface waters, most T&O outbreaks have never been forecast or partially traced to their biological origins. The scientific literature is well-documented about the role and the implication of both heterotrophic and autotrophic prokaryotes in the production of T&O compounds but very few studies have shown a direct relationship with environmental factors under natural conditions. The research presented in this document is an attempt to understand the dynamics of MIB and geosmin compounds, *'from source to sink'*, in a eutrophic drinking water supply reservoir, Eagle Creek Reservoir, located in central Indiana that experiences frequent annual T&O outbreaks. In the following chapters, we will first explore what environmental factors are important triggers for the production of MIB and GSM. Then, with the help of an

innovative high throughput sequencing (HTS) technology and augmented online databanks of 16S rRNA gene sequences, we will identify on one hand what bacteria are involved in the biosynthesis of these odorous metabolites and, on the other hand, which ones play a crucial role in their biodegradation. Finally, as there is currently no knowledge about the fate of these two terpenoids once released into raw water, naturally released after cellular death or chemically after application of an algaecide, our investigations will focus on the sediment-water interface. Sediment materials are not pristine surfaces but likely colloid-bound with humic, fulvic acids and other organics. This NOM is commonly found at concentrations parts per millions (mg/L) in natural raw waters and usually occurs at several orders of magnitude higher concentrations than MIB and GSM (ng/L), thus outcompeting T&O metabolites for adsorption sites [*Newcombe et al.*, 2002]. Therefore, in the last chapter, sorption and biodegradation experiments will be carried out to determine the role of bottom sediments in the removal/ sequestration of bacterial odorous terpenoids.

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CHAPTER 2 - INFLUENCE OF ENVIRONMENTAL FACTORS ON OFF-FLAVOR METABOLITE PRODUCTION BY BACTERIA IN A EUTROPHIC RESERVOIR

'Make hay while the sun shines'
Traditional

Introduction

Continental freshwater systems have received increasing scientific attention over the past decades as water quality deterioration and cyanobacterial bloom activity has been linked to eutrophication and global warming [*Paerl et al.*, 2001; *Paerl and Huisman*, 2009; *Shatwell et al.*, 2008]. When excessive nutrient is supplied to surface waters and temperature is optimal for growth, Cyanobacteria rapidly form massive water blooms [*Dokulil and Teubner*, 2000] which produce small dissolved organic compounds frequently involved in ecological [*Christoffersen*, 1996; *Miguéns and Valério*, 2015], economical [*Dodds et al.*, 2008; *Steffensen*, 2008] and health issues [*Carmichael*, 2001]. Such compounds can also support the growth of heterotrophic bacteria and shape the structure of the bacterioplankton community [*Eiler and Bertilsson*, 2004; *Louati et al.*, 2015]. Occurrences of off-flavor compounds synthetized by aquatic bacteria are a nuisance in source water systems and numerous episodes of taste-and-odor (T&O) compounds have been reported worldwide [*Juttner and Watson*, 2007; *Krishnani et al.*, 2008b].

In natural environments, two smelling terpenoids, geosmin (GSM; trans-1,10-dimethyl-trans-9-decalol) and 2-methylisoborneol (MIB), are the main metabolites causing T&O problems in drinking water [*Lanciotti et al.*, 2003; *Ma et al.*, 2013; *Watson et al.*, 2008; *Westerhoff et al.*, 2005]. They impart an earthy (GSM) and musty (MIB) taint to the water [*Izaguirre and Taylor*, 2004a] and to fish in aquaculture [*Klausen et al.*, 2005; *Robin et al.*, 2006]. Due to their lipophilic properties, both MIB and GSM easily cross the gills and guts of fish causing longer depuration or purging times for the removal of the earthy/moldy flavors accumulated in fish flesh prior to commercialization [*Burr et al.*, 2012; *Davidson et al.*, 2014; *Howgate*, 2004; *Reineccius*, 1991]. Each compound exists as (+) and (-) enantiomers but biological sources produce the (-) stereoisomer [*Krasner*, 1988]

which is ten times more potent than its (+) counterpart [*Juttner and Watson*, 2007]. Both MIB and GSM are potent odorous metabolites and have very low odor threshold concentrations (OTCs; at parts per trillion levels or ng L^{-1}) that can be detected by a human's olfactory senses [*Peter and Von Gunten*, 2007]. Drinking water quality, and thus the value of that water, are impacted by frequent occurrences of MIB and GSM in water supplies [*Davies et al.*, 2004; *Srinivasan and Sorial*, 2011]. The tertiary alcohol structure of both GSM and MIB render them extremely resistant to oxidation processes commonly used in water purification. Low concentrations tend to persist in finished water as conventional water treatment processes such as air stripping [*Terashima*, 1988], dissolved air flotation (DAF) [*Hargesheimer and Watson*, 1996], flocculation/ sedimentation/sand filter [*Hargesheimer and Watson*, 1996], oxidation with chlorine (Cl_2), chloramines and chlorine dioxide (ClO_2) [*McGuire*, 1999; *Nerenberg et al.*, 2000] or potassium permanganate ($KMnO_4$) [*McGuire*, 1999] fail to remove them entirely. Ozone (O_3) remains the strongest oxidant to efficiently remove MIB and geosmin but their oxidation can generate by-products such as low molecular weight ketones that also have odorous properties [*Lundgren et al.*, 1988; *Mcguire and Gaston*, 1988]. Besides the offensive odorous properties in source, recreational and drinking waters, T&O compounds currently have no regulations in the U.S. because they are associated with no known adverse effects on human health [*Dionigi et al.*, 1993]. Therefore, the U.S. Environmental Protection Agency (EPA) has defined no maximum concentration level (MCL) or maximum concentration level goal (MCLG) for MIB and GSM in drinking water.

The most important source of GSM and MIB in surface waters are bacteria [*Juttner and Watson*, 2007; *Watson*, 2003]. Both compounds are secondary metabolites synthetized through the isoprenoid pathway [*Bentley and Meganathan*, 1981] but their biological functions have not been elucidated. Trace concentrations of various odorous metabolites produced by bacteria may change the organoleptic properties of water and act as chemical attractants or repellents in the aquatic food-web for invertebrates, fish and humans [*Höckelmann et al.*, 2004; *Juttner and Watson*, 2007; *Watson et al.*, 2007]. In freshwater environments, Cyanobacteria have been known as the major producers of odorants [*Juttner and Watson*, 2007; *Watson*, 2010; *Watson et al.*, 2008]. Off-flavor compounds, such as MIB and GSM, which deteriorate the quality of water, are often associated with seasonal

blooms of *Oscillatoria, Anabaena flos-aquae, Planktothrix* and *Microcystis aeruginosa* [*Hayes and Burch*, 1989; *Li et al.*, 2007; *Su et al.*, 2015], where decaying blooms can release many odorous metabolites [*Ma et al.*, 2013; *Smith et al.*, 2008] and other bioactive compounds like cyanotoxins [*Smith et al.*, 2008]. Actinobacteria were the first identified organisms as producers of T&O metabolites [*Gerber*, 1979; *Gerber and Lechevalier*, 1965; *Juttner*, 1990; *B. Zaitlin and Watson*, 2006], and are very frequently found in limnetic systems [*Glöckner et al.*, 2000; *Methé and Zehr*, 1999; *Van der Gucht et al.*, 2005] and in bottom sediments [*Boucher et al.*, 2006; *Hahn et al.*, 2003], where they play a significant role in organic matter degradation [*Jiang and Xu*, 1996; *Johnston and Cross*, 1976; *Zaitlin et al.*, 2003]. Other organisms, such as Myxobacteria, also have the ability to synthesize GSM and MIB [*Dickschat et al.*, 2005; *Dickschat et al.*, 2007].

The environmental factors triggering the synthesis of geosmin by Actinobacteria were studied by *Wood et al.* [1983]. Some of the relevant factors were elevated nutrient levels in water, aerobic conditions and accumulation of sediment in the reservoir. The importance of nitrogen in the synthesis of geosmin by Actinobacteria was later confirmed [*Lind and Katzif*, 1988]. In the cyanobacterium *Fischerella muscicola*, *Wu and Jüttner* [1988] showed that geosmin was indifferently obtained under aerobic or anaerobic conditions, and that geosmin production was minimal at the optimal growth temperature but maximal at the lowest and highest temperature ranges. The influence of light and nutrient (N and P) on the synthesis of geosmin by *Oscillatoria brevis* was demonstrated to have no direct effect [*Naes et al.*, 1985]. Instead, it was concluded that geosmin detection was the result of increased algal biomass due to excess nutrient conditions rather than increased production rates [*Wnorowski*, 1992].

In central Indiana, Eagle Creek Reservoir has a long history of T&O events with major outbreaks of MIB and/or GSM occurring during the spring and the fall seasons. The main objective of the current study is to determine whether the reservoir hydrology drives the bacterioplankton communities leading to the production of T&O compounds and subsequently, to determine which bacterial taxa may be involved in the *in situ* production when the reservoir's water column is mixed.

Materials and Methods

Study site

Eagle Creek Reservoir (39°51’20”N, 86°17’39”W) located in central Indiana (Figure 2.1), receives drainage from 419.6 km^2 of the Eagle Creek Watershed and has a surface area of 5.7 km^2. The reservoir was constructed in 1967 to provide flood control and then drinking water for the city of Indianapolis and surrounding communities. The maximum depth ranges from about 11 to 13 meters, with the deepest areas located in the southern basin, near the dam. Eagle Creek Reservoir is a small, dimictic, and eutrophic water body with seasonal thermal stratification from June to September. Reservoir mixings usually occur in April/May and October each year. The mean annual discharge of Eagle Creek, the main tributary, is 35.74 $m^3.s^{-1}$ with maxima recorded between April and June. The calculated residence time of the reservoir is 39.5 days.

Figure 2.1: Sampling site location (dot) on Eagle Creek Reservoir. KEYE = Eagle Creek Airpark (square) where weather data were retrieved.

Sample collection and processing

Water samples were collected on a biweekly basis from mid-May to end of October 2013 near the dam where the strongest water column stratifications occur (Figure 2.1). Discrete water samples were collected with a vertical Van Dorn sampler at four different depths corresponding to sub-surface (0 m), summer epilimnion (3 m), metalimnion (6 m) and hypolimnion (9-10 m), *i.e.* 1 meter above the water-sediment interface. The photic zone was measured by the mean of a Secchi disk (SD), and the euphotic depth (Z_{eu}, in meters) was estimated from Secchi disk reading (Z_{SD}) using the relationship: Z_{eu}= 2.7 x Z_{SD} [*Tedesco and Clercin*, 2010]. Transmission of Photosynthetically Active Radiation (PAR, μmol photons m^{-2} s^{-1} from 400 to 700 nm) was measured at 50 cm intervals from just below the surface down to a depth of 1% incident PAR with a LI-192SA Underwater Quantum Sensor (LI-COR Inc., Lincoln, NE, USA). Prior to water collection, a submersible multi-parameter V2-6600 YSI probe (YSI, Inc., Yellow Spring, OH) was deployed in order to characterize the water column at meter intervals from the water surface down to the bottom. Measured parameters were water temperature (Temp, °C), conductivity (COND, μS cm^{-1}), total dissolved solids (TDS, g L^{-1}), dissolved oxygen (DO, mg L^{-1}), pH (*s. u.*), oxidation reduction potential (ORP, mV) and, chlorophyll and phycocyanin fluorescence (RFUs, Relative Fluorescence Units). The intensity of the reservoir thermal stratification was assessed by calculating the Relative Thermal Resistance to Mixing (RTRM) between adjacent layers (1 meter increment) within the water column. RTRM values were computed from temperature profile data using the relation [*Wetzel*, 2001]: $\psi=(\rho_{z2}- \rho_{z1})/(\rho_4- \rho_5)$; where ψ is the RTRM value (dimensionless), ρ_{z1} and ρ_{z2} are water densities at depths z_1 and z_2, respectively (kg m^{-3}) and ρ_4 and ρ_5 are water densities at 4 and 5 °C, respectively. Greater density differences between water layers are highlighted by higher RTRM values. Boundaries of the metalimnion are identified by RTRM 30 while the maximum value of RTRM identifies the depth of the thermocline. When RTRM values exceed 80, reservoirs are characterized as being “strongly stratified” [*Vallentyne*, 1957].

Nutrients and taste-and-odor compounds

Aliquots of the water sample were stored in 1L white HDPE bottles for nutrient analyses. Samples for methylisoborneol (MIB) and geosmin (GSM) analysis were stored in brown amber glass jars with no headspace or bubbles to avoid the volatilization of these compounds. All samples were stored on ice for transport to the laboratory. Inorganic nitrogen forms (nitrate, nitrite) were measured by ion chromatography Dionex DX-500 using the EPA 300.0 method [*USEPA*, 1993a]. Total Kjeldahl Nitrogen (TKN) was determined by digestion, followed by ammonia determination by ion selective electrode [*USEPA*, 1993b]. Total P was measured by ascorbic acid colorimetric method [*USEPA*, 1974]. Geosmin and MIB concentrations in water were quantified by a Head-Space Solid-Phase Micro-Extraction (HS-SPME) combined with a Gas Chromatography-Mass Spectrometry (GC-MS) to analyze the volatile metabolites MIB and geosmin according the Standard Method SM 6040D [*APHA*, 2000].

Enzyme-Linked Immuno-Sorbent Assay (ELISA)

Water sample aliquots were put in acid washed 125 mL clear glass for microcystin analysis using the ELISA analytical method which is sensitive for low levels of microcystins [*Pyo et al.*, 2005] in raw water; the limit of detection (LOD) of this method is 0.15 µg L^{-1} (ppb). Three freeze/thaw cycles followed by sonication (15 minutes at 40 kHz) was used to optimize the extraction of cyanotoxins. An aliquot (1 mL) of each sample was used for total microcystins analysis using competitive ELISA kits [*Fischer et al.*, 2001] targeting the non-proteinogenic amino acid (ADDA) found in cyanobacterial toxins following the protocols supplied by the manufacturer (Abraxis LLC., PA, USA). All assays were performed in duplicate. For statistical and graphical purposes, half of the LOD value was used to represent non-detected concentrations of microcystins in water [*Croghan and Egeghy*, 2003].

Hydrology and weather data

Stream discharge data from Eagle Creek, the main tributary of the reservoir, was recorded by the USGS super gage (USGS 03353200), located upstream from the reservoir

in Zionsville, IN. Weather data were recorded by Eagle Creek Airport (Figure 2.1, KEYE), adjacent to the reservoir.

Bacterial community identification

To identify and to determine the abundance of bacteria in water samples, a 460 bp-long amplicon was amplified by polymerase chain reaction (PCR). The gene-specific sequences target the 16S rRNA V3 and V4 regions (MiSeq v.3 Nextera XT, Illumina) using 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTAT CTAATCC as forward and reverse primers, respectively. The 16S rRNA sequencing provides reliable information on the taxonomic composition and the phylogenetic structure of natural bacterial communities expressed as Operational Taxonomic Units (OTUs). After collection near the dam, all discrete water column samples (0m, 3m, 6m and 10m) were put on ice in autoclaved 1-L HDPE brown bottles and filtered in the lab through 0.22 µm mesh size pores on a sterile glass filtration unit, then frozen for storage in 15-mL Falcon tubes. Samples were later shipped to Illumina, Inc., San Diego, CA for analysis on frozen filters to determine the bacterial community assemblages using 16S.

Statistical analysis

The 16S dataset along with physical and chemical parameters collected during the sampling campaign were analyzed with PAST 3.1 software [*Hammer et al.*, 2001]. We used Spearman's *rho* correlations to assess potential links between OTUs and off-flavor metabolite concentrations, and Canonical Correspondence Analysis (CCA) to extract major gradients among all physicochemical parameters that could trigger the growth of bacteria and the production of metabolites.

Results

Metabolite detections in raw water

On a routine basis, the local water company analyzes Eagle Creek Reservoir's raw water samples for odorous compound detections at several locations: at the dam, at the

water intake and before the entry of the water treatment plant. Two or three analyses are run weekly and the sampling frequency is usually augmented when either MIB or GSM levels exceed 10 ng L^{-1}. In 2013, a total of 359 water samples were analyzed; with an annual average of 10.8 and 12.6 ng L^{-1} for MIB and GSM respectively (Figure 2.2). Highest concentrations are commonly found between the months of April and June but maxima are reached during May for each metabolite (Table 2.1). It is noticeable that MIB concentrations exceed the odor threshold value of 10 ng L^{-1} seasonally during the spring and early fall while GSM detections are always above 4 ng L^{-1} but show minima throughout the summer months. GSM exceeds its odor threshold concentration 100% of the times (Table 2.1) from October to February and peaks during the month of May. Maximal values of MIB and GSM were both observed in May 2013 with 111.79 and 77.26 ng L^{-1} respectively.

16S rRNA analysis

Genetic data for the 2013 sampling campaign illustrated Cyanobacteria as a critical part of the bacterioplankton community but there are other orders distributed with depth (Figure 2.3). Proteobacteria as the second largest group includes a wide range of organisms but none of them are known producers of T&O compounds. Actinobacteria are known producers of T&O compounds and were found in significant abundance in the system at all depths. On average, Cyanobacteria represent 36% of the bacterioplankton community, followed by Proteobacteria (25%), Actinobacteria (7%) and all other groups individually lower than 5%. A spatial and temporal representation of the 16S rRNA dataset throughout the water column is presented in Figure 2.4, where the T&O outbreak (and algaecide intervention event as the white arrow) is compared to cyanobacterial (Oscillatoriales and Nostocales) and Actinobacterial (Acidimicrobiales and Actinomycetales) orders.

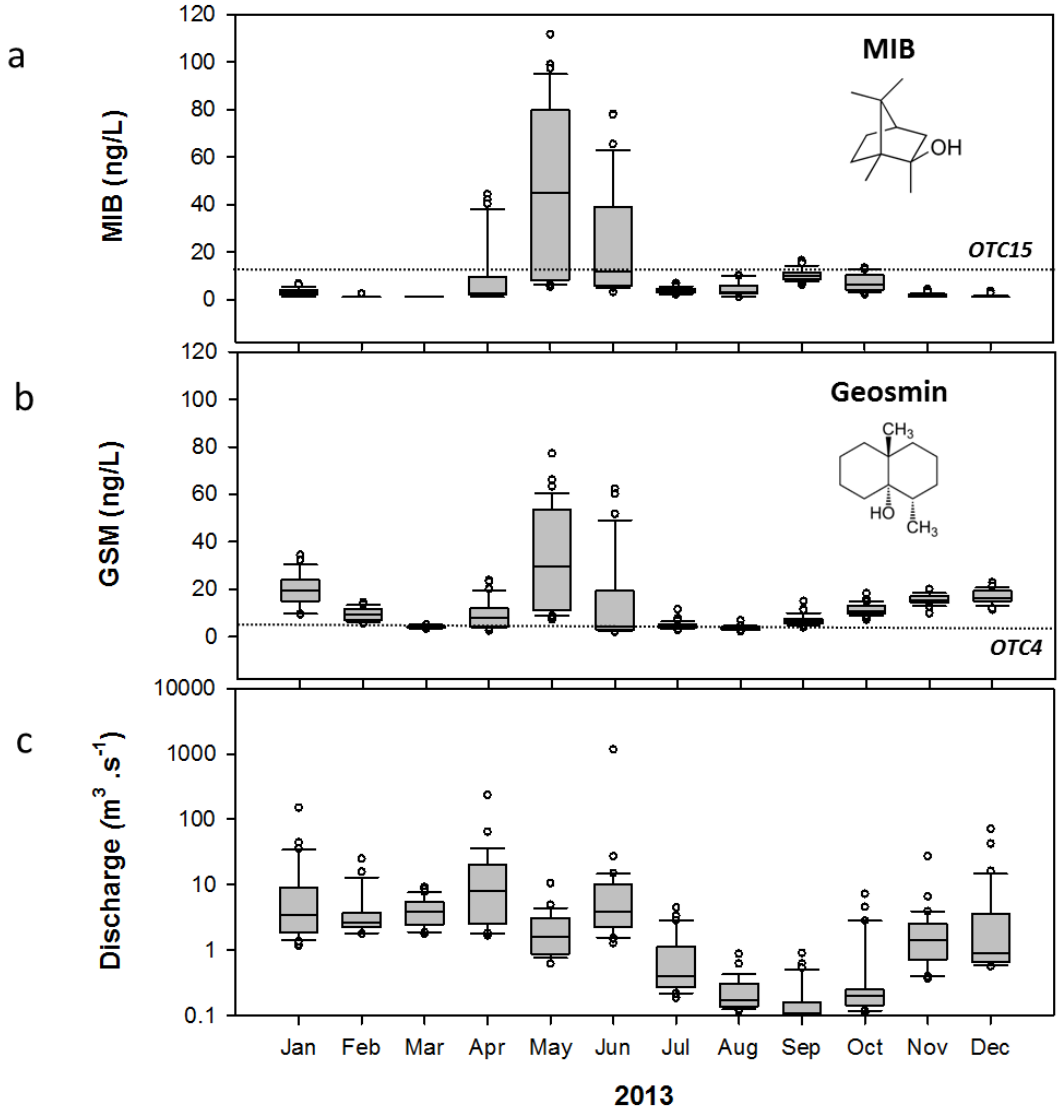

Figure 2.2: Monthly concentrations of a) 2-methylisoborneol (MIB), b) geosmin (GSM) and c) Eagle Creek discharges for the year 2013. Odor Threshold Concentrations (OTC; dotted lines) are 15 ng. L^{-1} for MIB and 4 ng. L^{-1} for GSM after *Peter and Von Guten* [2007]. Stream discharge is expressed in cubic meters per second ($m^{-3}.s^{-1}$).

Table 2.1: Monthly MIB and GSM concentrations (in ng L^{-1}) in Eagle Creek Reservoir; N= 359 samples and, %> OTC as percent of Odor Threshold Concentration exceedance indicating the percentage of samples collected that measured above the odor threshold. OTC values are defined by *Peter and Von Guten* [2007].

		MIB			**GSM**		
2013	n	Mean	Max	%> OTC	Mean	Max	%> OTC
Jan	25	3.16	6.73	0.0	19.59	34.44	100.0
Feb	27	1.05	2.48	0.0	9.36	14.49	100.0
Mar	23	1.00	1.00	0.0	4.17	5.18	60.9
Apr	31	9.01	44.32	22.6	9.03	23.83	80.6
May	38	44.84	111.79	60.5	32.10	77.26	100.0
Jun	38	23.97	78.13	57.9	13.31	62.27	55.3
Jul	31	3.69	6.79	0.0	4.83	11.57	74.2
Aug	31	4.37	10.19	3.2	3.61	6.86	29.0
Sep	33	10.12	16.44	36.4	6.87	15.00	97.0
Oct	30	7.25	13.37	30.0	11.51	18.23	100.0
Nov	28	1.50	4.39	0.0	15.60	20.11	100.0
Dec	24	1.18	3.59	0.0	16.90	22.91	100.0

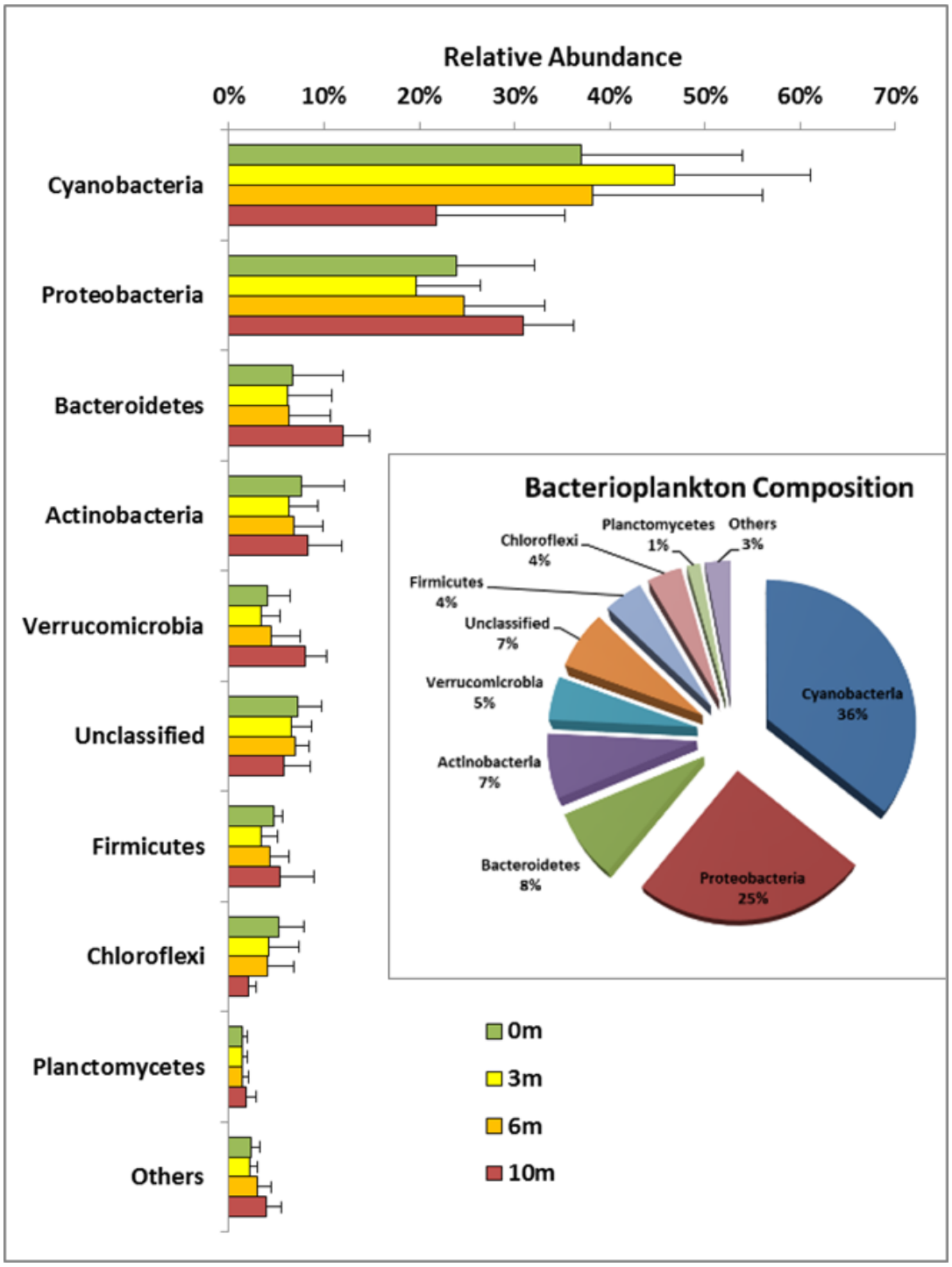

Figure 2.3: Average relative abundance of 16S reads for major clades of bacteria during 2013 campaign. Inset pie chart represents the average abundance for sub-surface samples.

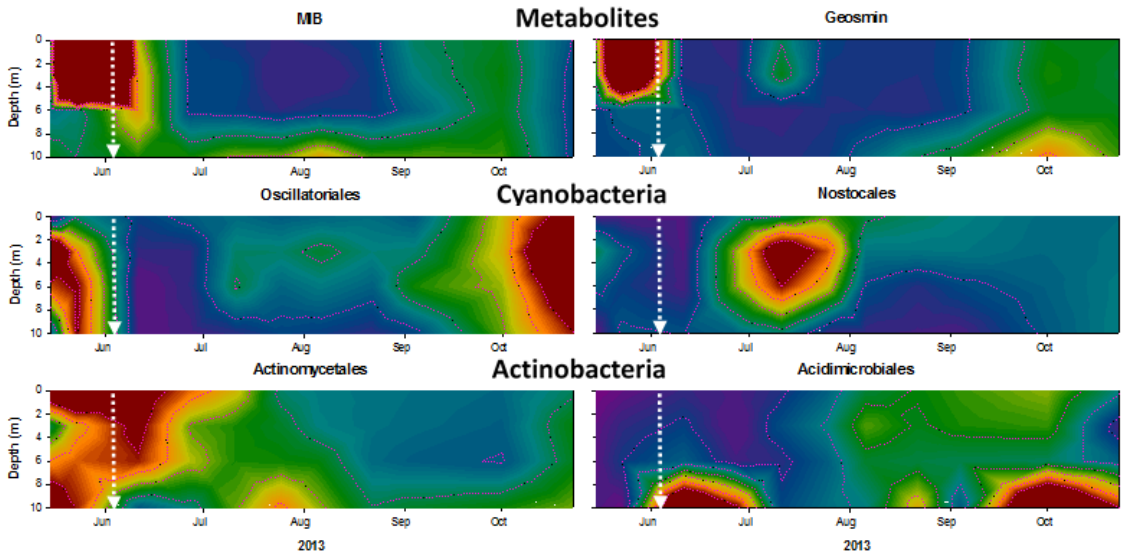

Figure 2.4: Spatial and temporal distribution of odorous metabolites and main bacterial orders throughout the water column. Top row: MIB and Geosmin; middle row: Cyanobacteria; bottom row: Actinobacteria; vertical dotted arrow: algaecide treatment date. Warmer colors represent highest concentrations or relative abundances. White arrow indicates timing of the June 2, 2013 algaecide application.

Identification of taste-and-odor producers

When the 16S rRNA dataset for microbial populations is checked for correlation against measured MIB and Geosmin levels, significant correlations are found for specific Cyanobacteria for GSM and for specific Actinomycetales species for MIB. Geosmin production is linked to several cyanobacterial OTUs, but not positively correlated with any statistical significance to any actinobacterial OTUs (Table 2.2). MIB production is linked to Actinomycetales OTUs, but not positively correlated with any cyanobacterial OTUs (Table 2.2). Actinobacteria are known T&O producers usually associated with soil environments [*Juttner*, 1990], bottom sediments [*Sugiura and Nakano*, 2000] or suspended sediments [*Jensen et al.*, 1994] whereas many others have not been documented yet about their potential capability to produce volatile odorous compounds (Table 2.2). These results strongly suggest that MIB production in Eagle Creek Reservoir is not linked to Cyano-bacteria at all.

Table 2.2: Correlation between potential T&O compound producers found in ECR with measured concentrations of MIB and GSM. Correlations with strong statistical significance are in bold $p < 0.05$, *$p < 0.01$ and **$p < 0.001$.

	Order	Genera	MIB	GSM	Habitat	Metabolites	References
Cyanobacteria	Chroococcales	*Chroococcus*	-0.48*	0.07	Planktic	-	-
		Cyanobacterium	-0.27	0.37	Planktic	-	-
		Microcystis	-0.58**	-0.18	Planktic	-	-
		Prochlorococcus	-0.59**	-0.48**	Planktic	-	-
		Snowella	-0.23	0.16	Planktic	-	-
	Oscillatoriales	*Microcoleus*	-0.17	0.45*	Planktic	GSM	Izaguirre and Taylor (1995)
		Oscillatoria	-0.57**	-0.07	Benthic	MIB, GSM	Izaguirre *et al.* (1983); Suurnäkki *et al.* (2015)
		Phormidium	-0.15	0.42*	Benthic	GSM	Izaguirre and Taylor (1995)
		Planktothrix	-0.17	0.53**	Planktic	GSM	Kutovaya and Watson (2014)
	Pseudanabaenales	*Leptolyngbya*	-0.41*	0.06	Epiphytic	MIB, GSM	Wang *et al.* (2015); Watson *et al.* (2016)
		Limnothrix	-0.54**	0.07	Planktic	-	-
		Prochlorothrix	-0.46*	-0.01	Planktic	-	-
		Pseudanabaena	-0.59**	0.04	Planktic	MIB, GSM	Izaguirre *et al.* (1999)
	Nostocales	*Aphanizomenon*	-0.60**	-0.16	Planktic	GSM	Kutovaya and Watson (2014); Suurnäkki *et al.* (2015)
		Calothrix	-0.31	0.32	Epiphytic	GSM	Kutovaya and Watson (2014); Suurnäkki *et al.* (2015)
		Cylindrospermopsis	-0.45*	-0.02	Planktic	-	-
		Dolichospermum	-0.75**	-0.37	Planktic	GSM	Watson *et al.* (2016)
		Nostoc	-0.22	0.44*	Benthic	GSM	Taylor *et al.* (2006)

Table 2.2 *continued*.

	Order	Genera	MIB	GSM	Habitat	Metabolites	References
Actinobacteria	Actinomycetales	*Arcanobacterium*	0.27	-0.15	Soil	-	-
		Cryobacterium	0.44*	-0.05	Soil	-	-
		Demequina	0.33	-0.24	Soil	-	-
		Georgenia	0.40*	-0.15	Soil	-	-
		Mycobacterium	0.10	-0.20	Soil	-	-
		Nocardia	0.15	0.17	Soil	MIB, GSM	Zaitlin and Watson (2006)
		Rhodococcus	-0.06	-0.32	Soil	-	-
		Saccharomonospora	0.45*	-0.11	Soil	-	-
		Saccharopolyspora	-0.24	-0.13	Soil	MIB, GSM	Komatsu *et al.* (2008); Watson *et al.* (2016)
		Sanguibacter	0.41*	-0.06	Soil	-	-
		Streptomyces	0.42*	-0.13	Soil	MIB, GSM	Saadoun, Schrader and Blevins (1997)
		Streptosporangium	0.23	0.08	Soil	-	
	Acidimicrobiales	*Acidimicrobium*	-0.13	-0.01	-	-	-
		Acidithiobacillus	0.08	0.14	-	-	-

Distribution of T&O producers

Having determined the major microbial OTUs potentially involved with T&O production, the combination of this 16S dataset with detailed hydrological, physical and chemical data allows us to investigate how changing conditions on Eagle Creek may select for specific populations of organisms capable of, and actually generating, MIB and GSM. In order to determine the most important environmental factors involved in the growth of off-flavor metabolite-generating bacteria, a Canonical Correspondence Analysis (CCA; Figure 2.5) was used. This constrained ordination technique extracts major gradients among the multitude of environmental variables measured on the field that would explain the abundances of bacterial OTUs at a given time of collection. Axes represent linear combinations of all environmental variables maximally projected in a Euclidean space; with axis 1 explaining 38.83% and axis 2, 26.64% of total variance of the dataset. Each environmental variable (here, physicochemical parameters) are represented as vectors with arrowheads indicating the direction of the increasing gradients. Dots illustrate bacterial OTUs.

The cloud of bacterial taxa is well divided into two separate clusters; with cyanobacteria in the lower part of the horizontal axis (dashed box) and Actinobacteria in the upper part (full box). The dispersion of individual taxa is ruled by different environmental gradients and vectors crossing clusters of dots that are more significantly important for these given clusters than distant ones, in other words the longer vector lines pointing towards the boxes delineating the two broad OTU groupings of Actinobacteria and Cyanobacteria indicate the groups are selected by different physicochemical conditions. On the horizontal axis, environmental parameters describe separate habitats: summer stratification on the left hand driven by warmer temperature and reservoir mixing periods on the right hand driven by turbidity. Ellipses representing different environmental conditions are defined according to sampling dates and depths of collection (Figure 2.5B). Spring and fall mixing conditions are represented by the grey ellipse. The main odorous episode of MIB and GSM that occurred during the month of May 2013 in Eagle Creek Reservoir is highlighted by the shaded grey ellipse. The summer stratification of the water column is represented by a black ellipse; epilimnion (full line ellipse) with highest temps and nutrient-depleted and, hypolimnion (dotted line ellipse) with nutrient-rich and oxygen-

depleted (low DO). Most cyanobacteria tend to thrive under high water temperatures, strong water column stratification (high RTRM) are correlated to high phycocyanin (PC) concentrations although a few taxa are found in more mixed (low RTRM) and turbid waters (low light coefficients; k_T) with cooler temperatures. These latter cyanobacteria, either benthic (*Nostoc*, *Phormidium*, *Microcoleus*) or pelagic (*Planktothrix*), often co-occur along with higher detections of geosmin and chlorophyll a. In opposition, Actinobacteria are driven by high stream discharges (Q) coupled with elevated nitrate (NO_3) concentrations. Some actinobacterial OTUs are more closely related to high MIB detections under conditions closely similar to geosmin-producing cyanobacteria.

Discussion

Reservoir hydrology and T&O events

Our study on odorous events highlights the key role of hydrological drivers on the production of MIB and GSM metabolites by different bacterial groups. These findings have direct implications on the forecasting and the management of off-flavor occurrences in source waters and then, the optimization of MIB and GSM removal. Eagle Creek Reservoir is a dimictic water body and receives most of its water during the spring: in April, from snow melt and, in May/June thanks to rainfalls and thunderstorms. Peak discharges bring terrestrial materials from soil erosion in the upstream watershed and could introduce Actinobacteria as well into the reservoir [*Zaitlin et al.*, 2003]. The highest concentrations of MIB and geosmin compounds are observed in May when the reservoir water columns are fully mixed and turbid. Throughout the summer, very low detections of each metabolite are found when the reservoir stratifies.

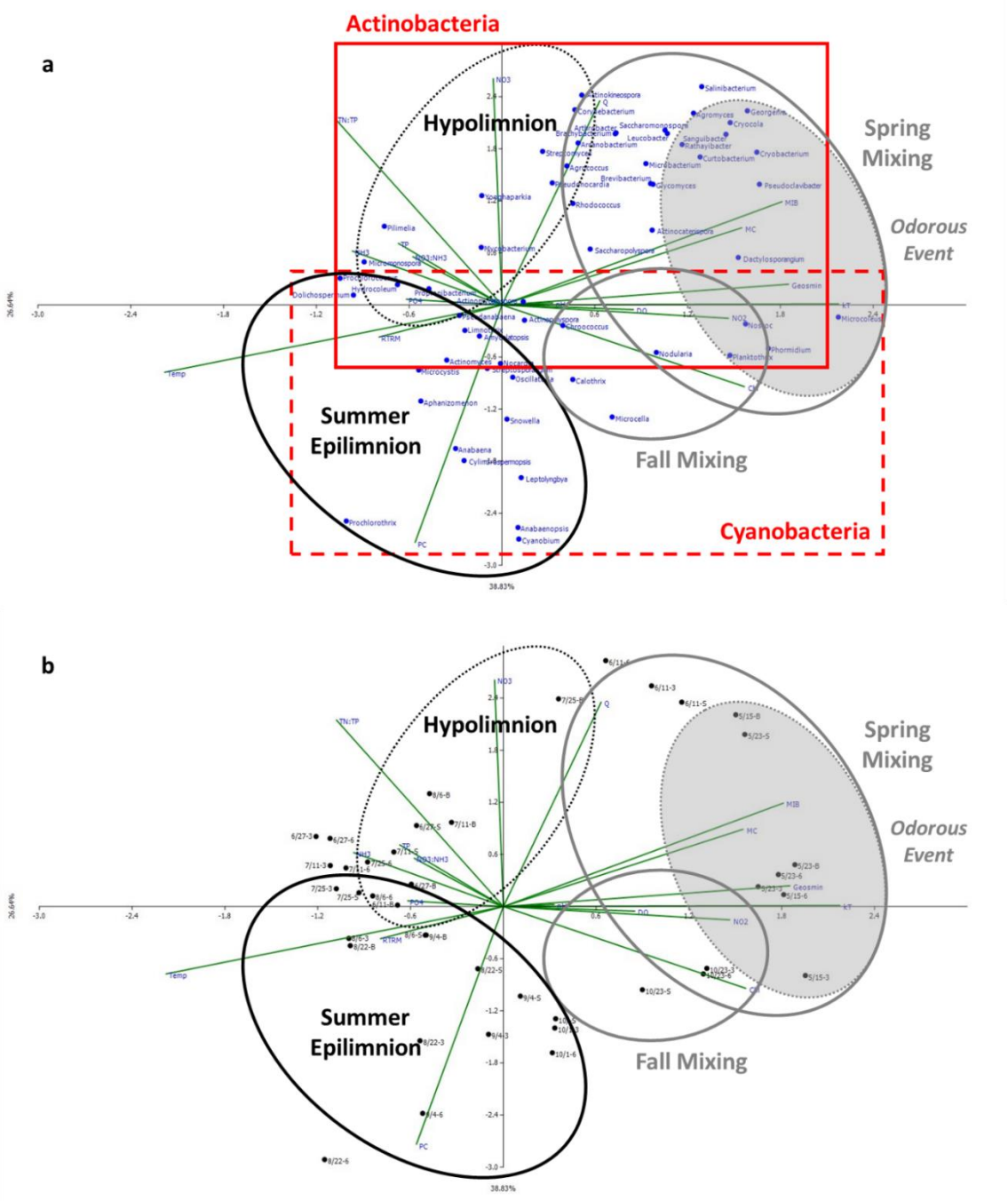

Figure 2.5: Canonical Correspondence Analysis showing: a) physical, chemical and genetic-based microbial data and, b) the distribution of 2013 campaign samples. Ellipses: mixed water column (grey), stratification (black), hypolimnion (black dotted), taste-and-odor event (shaded). Rectangles: Actinobacteria (solid line) and Cyanobacteria (dashed line). Blue dots represent bacterial OTUs and vectors are environmental parameters. Black dots are encoded as sampling dates – depths (S: surface; 3-meter; 6-meter and B: bottom).

The maxima of T&O compounds happen 37 days after a peak discharge of 236.7 $m^3 s^{-1}$ that occurred on April 29th 2013. A cross-correlation analysis of stream discharges versus metabolite concentrations recorded between April 1st and June 30th 2013 show a positive correlation of both MIB and GSM peaks with the inflow after a 37-day delay (Table 2.3). This delay value is closely similar to the reservoir's calculated residence time of 39.5 days and supports the hypothesis that external import and delivery of off-flavor-producing bacteria from the upstream watershed is part of the processes driving T&O production in this reservoir. However, during the months of April and May, average monthly retention times were shorter than the annual average; with 1.6 and 14.7 days respectively (Table 2.4). This implies than any imported bacteria would undergo a lag time prior to exponential growth and production of T&O compounds in the reservoir. Temperature plays a major role in bacterial growth but also in the production of metabolites [*Usha Kiranmayi et al.,* 2011]. Reservoir water temperatures in April/May are cool (<16.7ºC) and far from optimal growth temperatures for freshwater planktonic Actinobacteria; *i.e.* 25-35ºC [*Hahn and Pöckl,* 2005]. In temperate lakes, freshwater Actinobacteria have lower growth rates and lower optimal growth temperatures (0.34 h^{-1} and 28 ºC, respectively) compared to subtropical and tropical habitats (0.41 h^{-1} and 34 ºC) or culture media (0.6 h^{-1} and 35 ºC) [*Flowers and Williams,* 1977; *Hahn and Pöckl*, 2005]. The observed 37-day delay would provide enough time to support a slower growth rate of Actinobacteria and peak production of off-flavors metabolites as recorded by the end of May in Eagle Creek Reservoir.

Table 2.3: Cross-correlation between off-flavor metabolites (MIB, GSM) versus main tributary inflow (Q). Lags are expressed in days (d).

MIB			**GSM**		
Lag (d)	Correlation	*p*	Lag (d)	Correlation	*p*
-40	0.414	0.0493	-40	0.423	0.0443
-39	0.412	0.0454	-39	0.516	0.0099
-38	0.529	0.0065	-38	0.402	0.0461
-37	0.582	0.0018	-37	0.665	0.0002
-36	0.402	0.0376	-36	0.386	0.0466
-35	0.233	0.2322	-35	0.249	0.2015
-34	0.217	0.2671	-34	0.291	0.1329
-33	0.334	0.0820	-33	0.096	0.6265
-32	0.126	0.5139	-32	0.108	0.5783
-31	-0.049	0.7966	-31	-0.057	0.7653
-30	0.094	0.6146	-30	0.110	0.5559
…	…	…	…	…	…
-3	-0.107	0.4488	-3	-0.028	0.8457
-2	-0.216	0.1212	-2	-0.141	0.3153
-1	-0.223	0.1047	-1	-0.132	0.3420
0	-0.148	0.2825	0	-0.077	0.5781
1	-0.152	0.2671	1	-0.120	0.3815
2	-0.175	0.2000	2	-0.138	0.3167
3	-0.156	0.2564	3	-0.141	0.3041

Table 2.4: Mean monthly inflow discharge (Q, in cubic meters per second) and mean residence time (RT, in days) of Eagle Creek Reservoir, year 2013.

	Year	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**
N	365	31	28	31	30	31	30	31	31	30	31	30	31
Mean Q	52.0	124.7	44.6	42.3	211.4	23.2	72.9	9.2	2.4	1.8	7.5	25.7	58.7
Mean RT	39.5	2.7	7.7	8.1	1.6	14.7	4.7	37.0	139.5	193.3	45.8	13.3	5.8

Summer stratification and T&O occurrences

Epilimnion – Throughout the summer, the thermal stratification of the water column was strong and influenced the distribution of odorous metabolite-producing bacteria. The water column stratified from end of June to mid-September and some

geosmin production was observed in early July, likely due to the active growth of Nostocalean cyanobacteria *Dolichospermum sp.* in the epilimnion (a known Geosmin producer [*Watson et al.*, 2016]. The production of geosmin was maximal around the depth of 3 meters (Figure 2.4). No other detection of geosmin was found throughout the summer period.

Hypolimnion – Interestingly, the MIB signal was exclusively recorded in the hypolimnion of the reservoir, between 8 and 10 meters, whereas the upper parts of the water column was below detection (Figure 2.4). The 16S rRNA analysis documented the detection of Acidimicrobiales in June and again in late September and, Actinomycetales from June up to the end of October near the bottom. Actinobacteria are known to be prevalent and abundant in freshwater bottom sediment [*Schrader and Blevins*, 1993; *Sugiura and Nakano*, 2000] but the question of their survival under suboxic or anoxic conditions remains uncertain as abundances decrease with oxygen depletion [*Taipale et al.*, 2009]. Here, the 16S signature near the bottom simply does not necessarily distinguish between live and dead or senescent cells. Actinobacteria that spread out after the application of an algaecide occupied the whole water column when it was mixed during the month of June. When thermal stratification began to occur from early July, Actinobacteria progressively disappeared from the top layers of the water column and then were found near the bottom. Also noteworthy, the tailing of MIB detections can be observed near the bottom whereas no geosmin was recorded in the hypolimnion. Most of bacterial OTUs found in the hypolimnetic zone are identified as Actinobacteria, specifically: the anaerobic cellulolytic *Micromonospora* [*Leschine et al.*, 1988], the saprophytic and potentially pathogenic *Mycobacterium* [*Kazda*, 2010], the humic acid-reducing *Propionibacterium* [*Benz et al.*, 1998], and the alkaline soil indicator *Yonghaparkia* [*Yoon et al.*, 2006]. As none of these taxa are documented as potential off-flavor metabolite degraders, the recorded hypolimnetic MIB signal could simply result from cellular release after bacterial breakdown, with MIB remaining non-degraded afterwards. MIB concentrations that mimicked this Actinobacteria pattern thus reflect the release of dissolved metabolite into the bottom water after cellular death.

Distribution of T&O-producing bacteria

Reservoir hydrology is a critical driver influencing the spatial distribution of bacterial OTUs [*Šimek et al.*, 2011]. Seasonal mixing and thermal stratification create different habitats for bacteria; each having its own characteristics in term of light, pH, temperature, oxygen or nutrient gradients [*Ramsing et al.*, 1996]. To assess the influence of the reservoir hydrological cycle on the seasonal successions of bacterial OTUs, a Canonical Correspondence Analysis (CCA) was used (Figure 2.5). For Eagle Creek Reservoir, this multivariate analysis illustrates the seasonal succession of Actino- and Cyanobacteria that are correlated to the hydrological regime (Figure 2.5a). During the spring mixing (grey ellipse), numerous OTUs belonging to Actinobacteria are strongly correlated to high stream discharges (Q) and nitrates (NO_3). Vectors corresponding to off-flavor metabolites also point out to that direction; with MIB towards the cloud of Actinobacteria such as *Saccharopolyspora* [*Komatsu et al.*, 2008; *Watson et al.*, 2016] and *Streptomyces* [*Saadoun et al.*, 1997]; and GSM towards spring-blooming Cyanobacteria, such as *Microcoleus* [*Izaguirre and Taylor*, 1995], *Phormidium* [*Izaguirre and Taylor*, 2004b] and *Planktothrix* [*Kutovaya and Watson*, 2014]. This observed pattern grouping the cyanobacteria with GSM and the Actinobacteria with MIB confirms the results of the Spearman's *rho* test (Table 2.2). On the left side of Figure 2.5, the water column is more stable with higher water temperatures and higher stratification index values (RTRM). Under these conditions, the bacterial community is driven by summer Cyanobacteria that thrive in the epilimnion such as *Aphanizomenon spp. and Anabaena spp.* that can produce geosmin [*Kutovaya and Watson*, 2014; *Suurnäkki et al.*, 2015], *Cylindrospermopsis raciborskii*, *Anabaenopsis elenkinii* and *Prochlorothrix sp.* correlate with elevated phycocyanin signals throughout the summer period.

From late August, occurrences of other Cyanobacteria such as the benthic *Oscillatoria* [*Izaguirre et al.*, 1983; *Suurnäkki et al.*, 2015] and potentially the epiphytic *Leptolyngbya* [*Wang et al.*, 2015] may also have contributed to the production of geosmin although individual contributions to the general background signal is often very difficult to assess [*Juttner and Watson*, 2007]. As the water column de-stratifies, occurrences of both MIB and geosmin are observed throughout the water column. According to Figure 2.4, few Actinomycetales were present in the top layers of the water column in September

as the reservoir turns over. However, the presence of some Acidimicrobiales, *i.e. Acidimicrobium* and *Acidithiobacillus,* show no robust correlation with MIB or geosmin (Table 2.2). Meanwhile, the mixing-tolerant cyanobacteria *Planktothrix* (in the order Oscillatoriales, Figure 2.4) dominates the bacterial community. All co-occurring OTUs are known geosmin producers, *i.e.* the pelagic *Planktothrix* [*Kutovaya and Watson,* 2014], the benthic *Calothrix* [*Kutovaya and Watson,* 2014; *Suurnäkki et al.*, 2015] and *Nostoc* [*Taylor et al.*, 2006], and correlate positively to geosmin detections (Table 2.2). Increasing detections of geosmin were observed as the fall bloom of *Planktothrix* becomes more intense and severe in October.

Role of nitrogen

Taste-and-Odor Compounds – In Midwestern reservoirs, the production of secondary metabolites MIB and geosmin are likely to occur when the growth of potential producers is favored by low TN:TP < 30:1 (by mass) and low NO_3:NH_3 ratios [*Harris et al.,* 2016]. The CCA from Figure 2.5a shows that elevated abundances of Actinobacteria and Cyanobacteria are correlated to low TN: TP and NO_3: NH_3 ratios. Peaks of MIB and geosmin occurred during the spring when TN:TP and NO_3:NH_3 ratios were lower than 12 and 47 respectively; which is in concordance with Harris's results [*Harris et al.,* 2016]. A study in an Australian reservoir showed that occurrences of MIB were linked to increasing ammonia concentrations in water [*Uwins et al.*, 2007]. In the present study, although geosmin shows no correlation to inorganic nitrogen, MIB levels are strongly linked to ammonia in water (p <0.01; Table 2.5) consistent with Uwins' observations.

Actinobacteria – These bacteria have shown a positive correlation between nitrogen concentration and production of odorous metabolites [*Lind and Katzif,* 1988]. In Eagle Creek Reservoir, most Actinobacteria (Table 2.5) are strongly correlated to high levels of nitrate (*Arcanobacterium*, p <0.001; *Demequina*, p <0.001; *Rhodococcus*, p <0.001) and to ammonia (*Saccharomonospora*, p <0.05; *Streptomyces*, p <0.01). This supports the CCA results (Figure 2.5) that these OTUs occurred during high discharge periods in spring 2013 when nitrogen concentrations were maximal and then, supposedly the terrestrial origin of these bacteria from upstream watershed. While inorganic nitrogen may promote the growth of many Actinobacteria, *Streptomyces* a potent producer of MIB [*Saadoun et al.*, 1997] is

the only OTU in Eagle Creek Reservoir that shows a concurrent positive correlation to NH_3 (Table 2.5; *rho* = 0.45, p <0.01) and to MIB occurrences (Table 2.3; *rho* = 0.42, p <0.01). Although *Saccharomonospora* has a similar profile as *Streptomyces*, its growth is more likely due to the presence of high NO_3^- (p <0.001) in water rather than NH_3 (p <0.05). Additionally, its own MIB biosynthesis capacity has never been demonstrated.

Cyanobacteria – Conversely to Actinobacteria, the majority of Cyanobacteria are not correlated to nitrite levels or negatively correlated to nitrate and ammonia levels in the reservoir water (Table 2.5). Negative correlations are explained by the fact that most Cyanobacteria thrived in the epilimnion during the summer stratification when nitrogen was depleted. Non-heterocystous (Oscillatoriales, Pseudanabaenales) and heterocystous (Nostocales) Cyanobacteria also have the capacity to fix atmospheric nitrogen [*Bergman et al.*, 1997; *Fay*, 1992] and do not exclusively rely on the reservoir's nitrogen availability.

Table 2.5: Correlation between inorganic nitrogen (Nitrite, NO_2^-; Nitrate, NO_3^- and Ammonia, NH_3), major producing bacteria, off-flavor compounds MIB and GSM and, cyanotoxins (microcystins). Correlations with strong statistical significance are in bold with $p < 0.05$, $*p < 0.01$ and $**p < 0.001$.

Phylum	**Order**	**Genera**	**NO_2^-**	**NO_3^-**	**NH_3**	**Metabolites**
Cyanobacteria	Chroococcales	*Chroococcus*	0.25	0.02	**-0.37**	-
		Cyanobacterium	-0.05	**-0.53****	-0.29	-
		Microcystis	-0.11	-0.23	-0.27	-
		Prochlorococcus	-0.20	0.10	-0.09	-
		Snowella	-0.02	**-0.61****	-0.13	-
	Oscillatoriales	*Microcoleus*	0.26	-0.17	**-0.43***	GSM
		Oscillatoria	**0.31**	-0.14	**-0.33**	MIB, GSM
		Phormidium	**0.31**	-0.14	**-0.43***	GSM
		Planktothrix	0.27	-0.10	**-0.45***	GSM
	Pseudanabaenales	*Leptolyngbya*	0.08	**-0.50****	-0.17	MIB, GSM
		Limnothrix	0.12	-0.24	-0.28	-
		Prochlorothrix	-0.10	**-0.62****	-0.03	-
		Pseudanabaena	0.03	-0.09	**-0.45***	MIB, GSM
	Nostocales	*Aphanizomenon*	-0.09	-0.21	**-0.33**	GSM
		Calothrix	0.24	-0.18	**-0.43***	GSM
		Cylindrospermopsis	-0.08	**-0.33**	**-0.42***	-
		Dolichospermum	-0.05	0.16	-0.27	-
		Nostoc	0.26	0.04	**-0.53****	GSM

Actinobacteria	Actinomycetales	*Arcanobacterium*	-0.12	**0.70****	0.08	-
		Cryobacterium	-0.13	**0.41***	0.09	-
		Demequina	0.07	**0.59****	0.23	-
		Georgenia	-0.10	**0.58****	0.21	-
		Mycobacterium	0.16	-0.20	0.29	-
		Nocardia	0.04	**-0.63****	0.18	MIB, GSM
		Rhodococcus	0.15	**0.71****	-0.18	-
		Saccharomonospora	0.05	**0.49****	**0.32**	-
		Saccharopolyspora	**0.33**	**0.59****	**-0.35**	MIB, GSM
		Sanguibacter	0.07	**0.58****	0.20	-
		Streptomyces	-0.12	0.00	**0.45***	MIB, GSM
		Streptosporangium	0.25	-0.35	0.11	-
Metabolites		MIB	-0.09	-0.22	**0.45***	
		GSM	0.16	-0.29	-0.08	
		Microcystins	-0.18	-0.04	-0.07	

Conclusions

In Eagle Creek Reservoir, recurring major T&O episodes are very frequently observed during the spring and the fall while fewer detections are recorded during the summer time. These odorous events usually occur after the reservoir has received inflows from its main tributary in April and May. Spring episodes of MIB and geosmin have in general longer durations and are more intense than any other times later in the year. High stream discharges bring in nutrients and mix the reservoir water columns. These conditions are favorable to support the growth of some Actinobacteria (*Streptomyces*) and Cyanobacteria (*Planktothrix*) that are involved in the *in situ* production of MIB and geosmin. In the present study, a lag phase of 37 days between a major peak discharge and highest detections of both metabolites in the reservoir waters was observed. This lag phase seems to represent the time required for Actinobacteria to be transported from the watershed to the reservoir, to adapt to a non-optimal growth temperature and then synthetize off-flavor metabolites. This information provides a useful clue for managers desiring to anticipate major odorous events and may want to disrupt the bacterial growth before it becomes severe. Geosmin was strongly linked to the presence of *Planktothrix* in the reservoir while MIB detections frequently occurred when *Streptomyces* was around.

As seen in Eagle Creek Reservoir, algaecide treatment was only effective against Cyanobacteria and disrupted the geosmin production whereas it had little impact on Actinobacteria and MIB which remained detectable in the water a couple of weeks after the treatment. This observation highlights a difference in the chemical and biological behavior of the two metabolites MIB and GSM which should influence the choice of decision makers before treating a water supply reservoir. Genetics remains an important tool while studying bacterial communities in aquatic environments. The 16S method can provide key insights regarding the presence of potential T&O-producing bacteria at a given time compared to traditional morphologically based microscope counting techniques that can miss information about species without morphological distinctiveness, such as the Actinobacteria.

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CHAPTER 3 – BACTERIOPLANKTON COMMUNITIES' COMPOSITION IN A EUTROPHIC RESERVOIR DURING OCCURRENCES OF TASTE-AND-ODOR COMPOUNDS MIB AND GEOSMIN

'Be fruitful and multiply and, replenish the earth'
Genesis I, v.28

Introduction

Over the past decades, rRNA-based technologies have promoted the study of the microbial diversity [*Griffiths et al.*, 2000; *Marchesi et al.*, 1998; *Rheims et al.*, 1996] and led scientists to refinements in their understanding of the uncultured and undiscovered microorganisms which were recognized as a major component of all bacterial communities [*Amann et al.*, 1995; *Hugenholtz et al.*, 1998]. With the development of the polymerase chain reaction (PCR) technique [*Pace et al.*, 1986; *Vosberg*, 1989], the inventory for bacterial taxa from any environmental sample was made possible without cultivation [*Giovannoni et al.*, 1990]. Most surveys were performed in marine systems to characterize picoplankton [*Giovannoni et al.*, 1995; *Schmidt et al.*, 1991] and microbial communities in soil environments [*Bruce et al.*, 1992; *Rondon et al.*, 2000] whereas there were very limited insights into what bacterial communities could look like in freshwater habitats; with the exception of the phylum of Cyanobacteria which could easily be grown on culture media [*Hugenholtz et al.*, 1998] and as morphologically distinct forms under the microscope [*Zapomělová et al.,* 2008]. Since the late 1990's, randomly cloning environmental DNA techniques, known as metagenomics [*Handelsman et al.*, 1998], have emerged and constantly add to existing 16S rRNA databases that illustrate the diversity of the microbial world. Meanwhile, scientists realize that the microbial diversity is much larger than they were able to estimate prior to the advent of molecular methods [*Pace*, 1997] and high throughput sequencing techniques [*Zarraonaindia et al.*, 2013].

The extensive use of these molecular tools in the exploration of microbial diversity has enabled the identification of bacterioplankton in freshwater ecosystems [*Eiler and Bertilsson*, 2004; *Newton et al.*, 2011; *Zwart et al.*, 2002]. The bacterioplankton communities' composition (BCC) holds a central role in aquatic food webs [*Pernthaler*,

2005], plays a significant role in carbon- and nutrient-cycling, and is involved in many biochemical processes [*Cotner and Biddanda*, 2002]. Through many studies, the BCC has been shown as greatly variable between different freshwater lakes [*Keshri et al.*, 2018; *Lindström*, 2000; *Van Der Gucht et al.*, 2001] and its growth is highly influenced by environmental factors such as geographical regions [*Lindström and Leskinen*, 2002], water temperature [*Keshri et al.*, 2018; *Pearce*, 2005], pH and water retention [*Lindström et al.*, 2005], nutrient loads [*Haukka et al.*, 2006; *Van der Gucht et al.*, 2005] and, potentially the lake trophic status [*Eiler and Bertilsson*, 2004; *Lindström*, 2000; *Yannarell et al.*, 2003]. The BCC of freshwater environments is typically dominated by Actinobacteria [*Keshri et al.*, 2018; *Tanaka et al.*, 2017], Bacteroidetes [*Schmidt et al.*, 2016; *Šimek et al.*, 2001], Cyanobacteria [*Ávila et al.*, 2016; *Su et al.*, 2017; *Woodhouse et al.*, 2016; *Zhao et al.*, 2016], or Proteobacterial bacterioplankton [*Olapade*, 2017; *Salmaso et al.*, 2017; *Wu et al.*, 2012]. The broad range of complex interactions between taxa within the bacterioplankton community, and between taxa with environmental factors, has been proposed using association networks [*Fuhrman*, 2009; *Steele et al.*, 2011].

Cyanobacteria are frequently involved in the production of bioactive compounds of concerns such as cyanotoxins and taste–and-odor compounds [*Carmichael*, 1992]. Worldwide reports show that various T&O compounds can be a source of nuisance in water bodies; these secondary metabolites during odorous outbreaks in drinking water supply reservoirs are caused by microbial growth [*Juttner and Watson*, 2007]. Unpleasant T&O occurrences of earthy/musty terpenoids such as geosmin (GSM) and 2-methyl-isoborneol (MIB) are common [*Watson et al.*, 2008; *Westerhoff et al.*, 2005]. In aquatic environments, the main source of T&O compounds are Cyanobacteria and Actinobacteria [*Juttner and Watson*, 2007; *Watson et al.*, 2008]. Deteriorating the water quality, T&O occurrences in source waters are often associated with seasonal blooms of Cyanobacteria [*Li et al.*, 2007; *Su et al.*, 2015] while Actinobacteria contributions are often neglected.

MIB was first characterized as a methylated monoterpene (C_{11}) alcohol and, geosmin as a degraded sesquiterpenoid (C_{12}) alcohol that has lost an isopropyl group (C_3), both derived from the biosynthesis of C_5 isoprene units [*Bentley and Meganathan*, 1981]. These compounds have relatively low molecular weights; *i.e.* 168.28 g. mol^{-1} for MIB (CAS #2371-42-8) and 182.31 g. mol^{-1} for geosmin (CAS #19700-21-1) [*Pirbazari et al.*,

1992], moderate solubility, and moderate hydrophobicity [*Song and O'Shea,* 2007]. Biosynthetically, terpenes are generated by terpene cyclases (=isoprenoid synthase type I) from linear precursors: geranyl diphosphate (GPP), the immediate precursor of C_{10} monoterpenes and, farnesyl diphosphate (FPP) of cyclic C_{15} sesquiterpenes [*Cane et al.*, 2006]. These terpene cyclase enzymes, *i.e.* the MIB synthase [E.C. 4.2.3.118] [*Wang and Cane*, 2008] and the germacradienol/geosmin synthase [E.C. 4.1.99.16] [*Jiang and Cane*, 2008] were both identified in the actinobacterium *Streptomyces coelicolor*. Biosynthesis of terpenes can follow two different pathways and both can be used by the same organism. The 2-methylerythritol-4-phosphate (MEP) pathway is considered to be prevalent in bacteria such as Actinobacteria and Cyanobacteria [*Kuzuyama,* 2002; *Lange et al.,* 2000; *Spiteller et al.*, 2002] whereas the mevalonate (MVA) pathway would be the preferred route by Myxobacteria, Archaea and Eukaryotes [*Boucher et al.,* 2004; *Dickschat et al.*, 2005] of the two isoprenoid precursors: isopentenyl pyrophosphate (IPP) and the dimethyl-allyl diphosphate (DMAPP) biosynthesis. However, there are exceptions as some bacteria do possess the MVA pathway [*Bochar et al.,* 1999].

The use of the metagenomics technique would allow to characterize the BCC, assuming that both T&O-producing Cyanobacteria and Actinobatceria cooccurred and were highly abundant during the odorous events of Eagle Creek Reservoir. Similarly, during those events, detections of key enzymes involved in the biosynthtetic pathways of MIB and GSM should be enhanced as the expression of the MIB and GSM synthase genes is increased as both odorous compounds were recorded in the reservoir water. The aim of the present work was to identify geosmin- and MIB-producing bacteria in Eagle Creek Reservoir using a shotgun sequencing approach in order to illustrate the connections between microbial organisms and environmental variables. Co-occurring bacteria during a T&O outbreak could reveal potential associations of species. The shotgun technique also allows to screen for metabolic enzymes involved in the biosynthetic pathways of geosmin and MIB. The recovery of key enzyme would unmask critical producers if the relationship is assessed.

Materials and Methods

Study site

Eagle Creek Reservoir (39°51’20”N, 86°17’39”W) located in central Indiana (Figure 3.1), receives drainage from 419.6 km^2 of the Eagle Creek Watershed (HUC 05120201120). The reservoir was constructed in 1967 to provide flood control and then drinking water for the city of Indianapolis and surrounding communities. The maximum depth ranges from about 11 to 13 meters, with the deepest areas located in the southern basin, near the dam. Eagle Creek Reservoir is a small, dimictic, and eutrophic water body with seasonal thermal stratification from June to September. Reservoir mixings usually occur in April/May and October each year. The mean annual discharge of Eagle Creek, the main tributary, is 35.74 $m^3.s^{-1}$ with maxima recorded between April and June. The calculated residence time of the reservoir is 39.5 days.

Figure 3.1: Sampling site location (black dot) near Eagle Creek Reservoir dam.

Sample collection and processing

Water collection – Water samples were collected in May, July and October 2013 near the dam where the strongest water column stratifications occur (Figure 3.1). Discrete water samples were collected with a vertical Van Dorn sampler at four different depths

corresponding to sub-surface (0 m), epilimnion (3 m), metalimnion (6 m) and hypolimnion (9-10 m), *i.e.* 1 meter above the water-sediment interface. A total of 11 samples was collected as the sub-surface sample from October did not recover enough genetic materials to be processed. A submersible multi-parameter V2-6600 YSI probe (YSI, Inc., Yellow Spring, OH) was deployed to measure water temperature (Temp, °C) and pH (*s. u.*) at each sampling depth.

Nutrients and T&O compounds analysis – Water sample splits were stored in 1-L white HDPE bottles for nutrient analyses and brown amber glass jars with no headspace or bubbles to avoid the volatilization of methylisoborneol (MIB) and geosmin (GSM). All samples were stored on ice for transport to the laboratory. Inorganic nitrogen forms were measured by ion chromatography using a Dionex DX-500 ion chromatograph applying the EPA 300.0 method [*USEPA*, 1993a]. Total Kjeldahl Nitrogen (TKN) was determined by digestion of organic nitrogen compounds with sulfuric acid, then followed by free-ammonia determination by ion selective electrode [*USEPA*, 1993b]. Total P was measured by the ascorbic acid colorimetric method [*USEPA*, 1974]. Methylisoborneol and geosmin concentrations were quantified by a Head-Space Solid-Phase Micro-Extraction (HS-SPME) combined with a Gas Chromatography-Mass Spectrometry (GC-MS) to analyze the volatile and odorous compounds [*APHA*, 2000].

Bacterioplankton community composition – After collection near the dam, all discrete samples (*i.e.* 0m, 3m, 6m and 10m) were put on ice in autoclaved 1-L HDPE brown bottles and filtered in the lab through 0.22 µm mesh size pores on a sterile glass filtration unit, then frozen for storage in 15-mL Falcon tubes. Samples were later shipped to Illumina, Inc., San Diego, CA for analysis on frozen filters to determine the phylogenetic structure of the BCC by next-generation sequencing (NGS) Shotgun Metagenomics method. Sequencing-ready libraries were prepared using the Prep Kits for MiSeq v.3 Nextera XT, Illumina. Data outputs were uploaded on the MG-RAST server [*Meyer et al.*, 2008] for k-mer alignments and taxonomic profiles; using the *Refseq* and *SEED Subsystems* databases for OTUs and hierarchical annotations. Cut-off values for abundance profiles of harvested reads were set to 10^{-5} (e-value), 70% identity and a minimal alignment of 15 nucleotides.

Measurement of sample biodiversity

In order to compare each sample's biodiversity and complexity, we used indices that combine richness and abundance. The simplest metric to measure biodiversity is the Specific Richness (S) that corresponds to the number of species present in the sample [*Whittaker*, 1972]. Shannon's diversity (*H'*) is commonly used for assessing species diversity between different habitats/ samples [*Hutcheson*, 1970]. Simpson's dominance (D) measures the probability that two random individuals from a sample belong to the same species. D index is simply the inverse of Simpson's original index [*Simpson*, 1949] and is commonly used to emphasize the diversity [*Magurran*, 2004]. Simpson's evenness (E) takes a value between 0 and 1, with 1 assuming that individuals are completely evenly distributed in the community [*Hill*, 1973]. Calculations of diversity indices are shown in Supplemental Table S1.

Sequence processing

A bacterioplankton abundance heat map was generated using the matrix visualization software Morpheus from the Broad Institute website (https://software.broadinstitute.org/morpheus/). Descriptive statistics, Non-metric Multi-dimensional Scaling (NMDS) and Correspondence Analysis (CA) were generated using the PAST 3.1 software [*Hammer et al.*, 2001]. For NMDS, sample dissimilarities derived from Bray-Curtis distances weighted on OTU abundances. The CA shows the relationships between OTUs and enzyme reads identified from the MG-RAST *SEED Subsystems* database.

Network construction

All 11 samples were used for the construction of a global network of bacterial OTUs interacting with environmental parameters using Cytoscape 3.6.0 software [*Shannon et al.*, 2003]. Only OTUs belonging to Actinobacteria and Cyanobacteria were illustrated and identified down to the genus level whereas all other bacteria were grouped up at the phylum level for visual clarity. Global and sub-sequent networks were generated using the '*Prefuse Force Directed and Unweighted*' and the '*Hierarchical Edge Bundling*' algorithms to reduce visual clutter of adjacent edges between parent nodes [*Holten*, 2006].

The relative abundances of each taxonomic groups were extracted in order to build a correlation matrix. Both correlation *r* and significance *p* matrices were generated by calculating all pairwise Spearman's rank correlation possibilities between all OTUs and, all OTUs versus environmental variables. For network constructions, only robust and significant correlations ($r > 0.6$ and $p < 0.05$; $r > 0.7$ and $p < 0.01$) were retained [*Barberán et al.*, 2012].

Results

Bacterioplankton diversity and seasonal variations

In Eagle Creek Reservoir, the Specific Richness (S) did not show any significant differences within the water column and throughout seasons (Figure 3.2A); with the lowest S (590 genera) near the bottom in fall and the highest S (599 genera) at 6 meters during the spring (Supplemental Table S2). The average Shannon's diversity (*H'*) index was equal to 3.63 for all seasons and depths combined; with both lowest (2.74) and highest (4.98) values found near the bottom during the fall and summer respectively (Figure 3.2B). Simpson's dominance (D) index increased with depth independently of the season. Highest values of D were found near the bottom of the water column and during the fall (Figure 3.2C). Reversely, Simpson's evenness (E) tended to increase with upper layers of the water column although the bottom layer in summer displayed the highest value while the reservoir was stratified (Figure 3.2D).

In each sample, harvested 16S rRNA reads were assigned to different taxonomic ranks from phyla to genera and used to determine the relative abundances of the top 5 most abundant phyla (Supplemental Table S3) and top 5 genera in each of these phyla (Figure 3.3). From spring to fall, Actinobacteria were the most abundant bacterial group in all samples. The highest and the lowest abundances of Actinobacteria were both recorded in the hypolimnion (10-m) during the spring (83.9%) and the summer (24.8%), respectively. Within the water column, Actinobacteria always represented more than 60% of the total bacterioplankton community except during the summer when they tend to be less abundant. The genus *Arthrobacter* is by far the most representative out of the 66 actinobacterial OTUs and its individual abundance exceeded more than 40% of the total bacterial community

during the fall in the whole water column (Figure 3.3). Proteobacteria were the second most abundant (Supplemental Table S3) with *Acidovorax* whose maximal abundance reached 1.03% at 3-m depth during the spring, being the most representative genus out of 271 OTUs. Then, Firmicutes with 99 OTUs and Bacteroidetes with 42 OTUs were represented by the genera *Bacillus* (>5% in the upper layer in spring and summer) and *Flavobacterium* (max. abundance 0.46% at 10-m in summer), respectively. Finally, Cyanobacteria always maintained the 5th position with less than 3% of the total bacterioplankton abundance. However, their abundance jumped to 7.5% near the surface during the summer (Supplemental Table S3). This increase was due largely to increases of the pico-cyanobacteria *Synechococcus* (Figure 3.3).

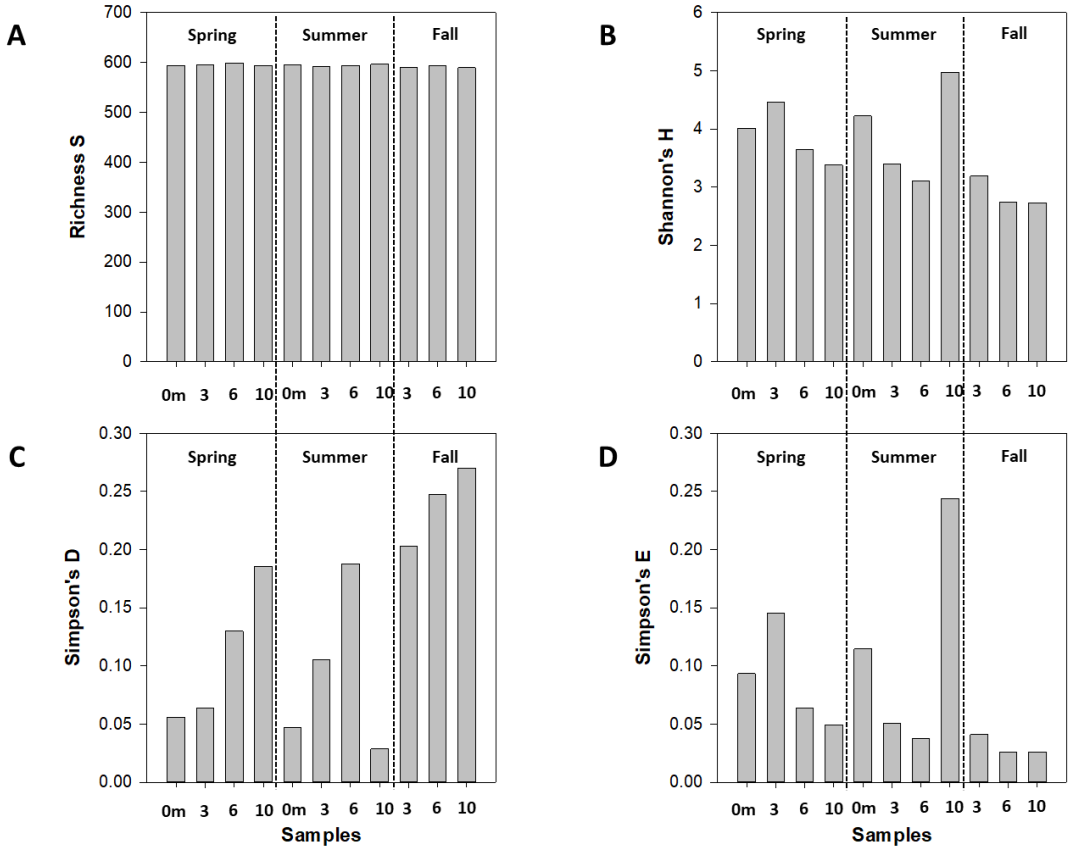

Figure 3.2: Spatial (depth: 0, 3, 6 and 10 meters) and temporal (seasonal) variations of the bacterioplankton community alpha-diversities in Eagle Creek Reservoir. A) Specific richness [S]; B) Shannon's diversity [H']; C) Simpson's dominance [D]; and, D) Simpson's evenness [E].

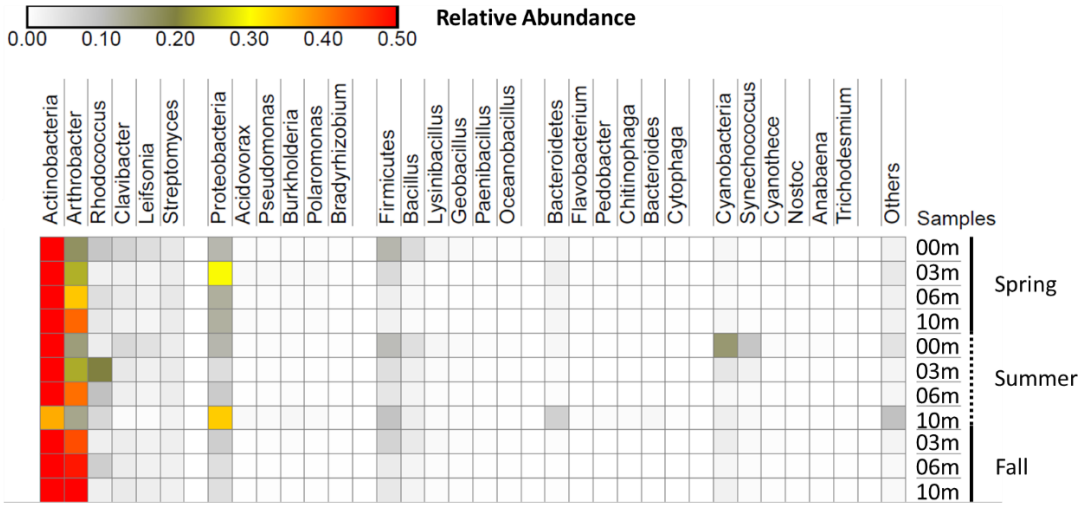

Figure 3.3: Heat map of the top 5 most abundant bacterioplankton phyla and their top 5 genera in Eagle Creek Reservoir discrete water samples.

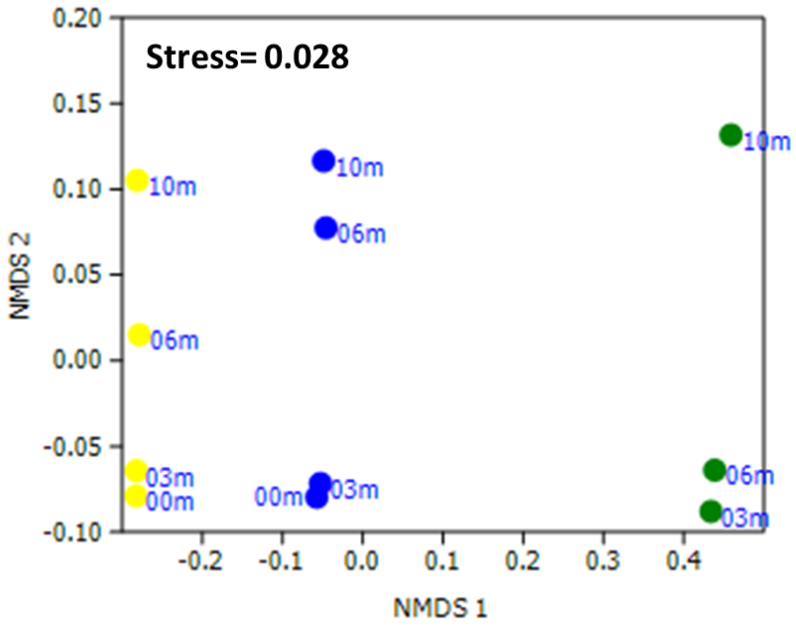

Figure 3.4: Non-metric multidimensional scaling (NMDS) plot illustrating the similitude of bacterioplankton assemblages among Eagle Creek Reservoir's 11 water samples. Seasons were represented with different colors such as spring (blue), summer (yellow) and fall (green).

These results suggest that the presence and the dominance of some bacterial OTUs are influenced by both its position within the water column and seasons. The different community assemblages of Eagle Creek Reservoir bacterioplankton were compared using a NMDS analysis. The NMDS analysis confirmed the well-separated and successive bacterioplankton communities along time (horizontal axis) and space (vertical axis) gradients (Figure 3.4). The BCC from sub-surface (00-m) and epilimnetic (03-m) samples are clustered and not very dissimilar in May and July, and between 03-m and 06-m in October compared to bottom samples. This highlights homogeneous water layers and, by extension, partially mixed conditions during the months of May and October and, a well stratified water column in July (Figure 3.4). The low stress value of 0.028 indicates the very good fit of the NMDS ($R^2 > 0.97$).

Seasonal variations of MIB and Geosmin

Eagle Creek Reservoir witnesses frequent T&O episodes of both MIB and geosmin annually. Usually, these odorous events occur during the spring and the fall; peaks in concentration are recorded in May and/or September. In 2013, MIB and GSM co-occurred during the month of May. Maximal concentrations of MIB and GSM reached 120.9 and 51.4 ng. L^{-1}, respectively (Supplemental Table S4). Such elevated levels of MIB and GSM were not recorded again and the month of May remained the major episode of T&O in the reservoir in 2013. Detections of the odorous compounds barely exceeded 20 ng. L^{-1} in October and minima were recorded in July.

Bacterial consortia and environmental parameters

Interactions of environmental variables on Eagle Creek Reservoir's BCC and interspecific relationships are represented by an association network where edges represent robust (r >0.6) and significant (p >0.05) positive correlations (full lines) and negative correlations (dotted lines). Most OTUs are clustered in four different groups based on species-species relationships defined by their direct first neighbors; thus forming two predominant bacterial consortia (CI and CII) and two peripheral minor ones (Ci and Cii; Figure 3.5).

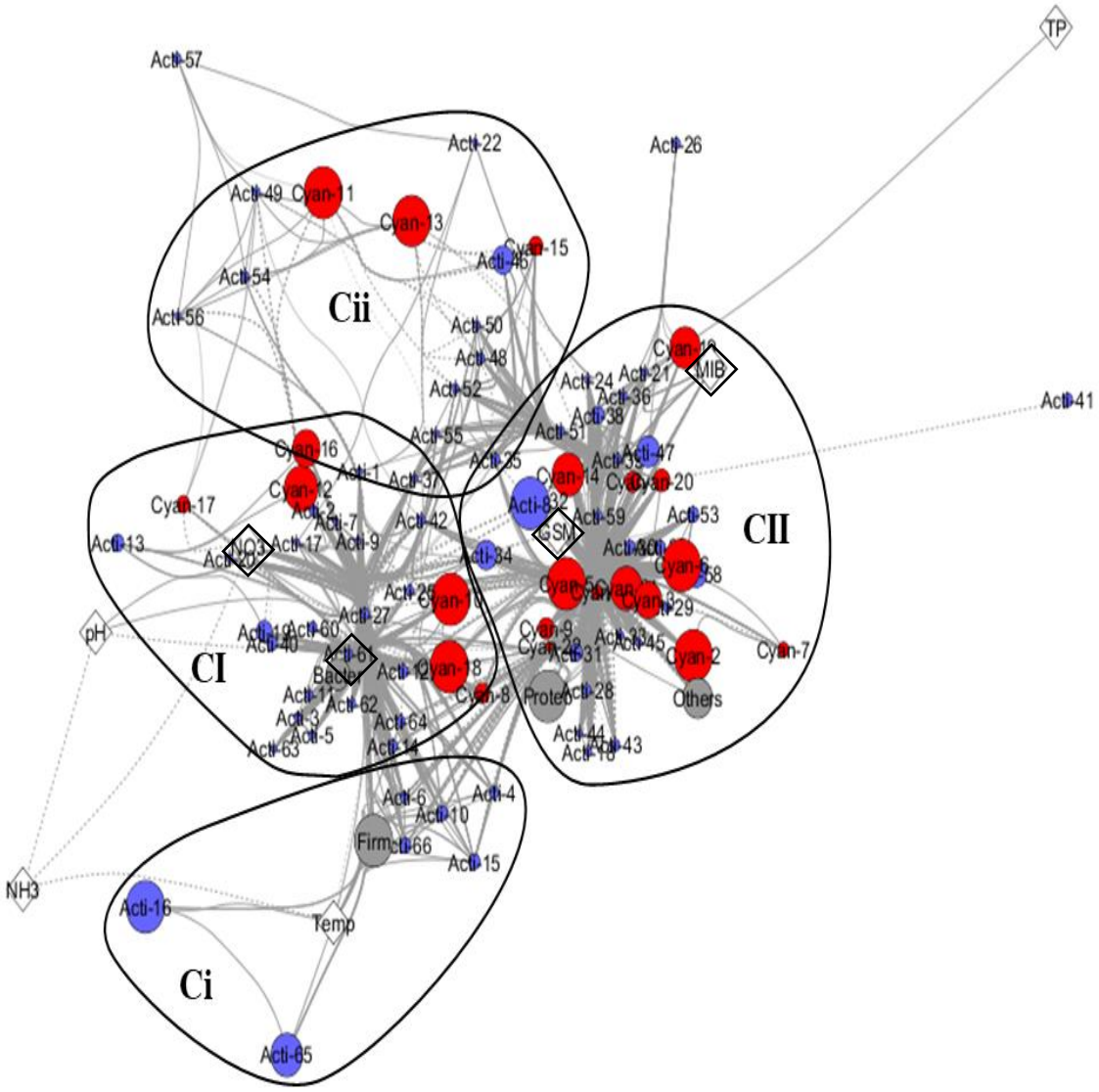

Figure 3.5: Global network of Eagle Creek Reservoir bacterioplankton communities' composition. Only correlations that are strong ($r > 0.6$) and statistically significant ($p < 0.05$) are shown by full gray lines (positive correlations) and dotted lines (negative correlations). Legend: diamonds) Environmental variables: GSM, geosmin; MIB, methylisoborneol; NH_3, ammoniac nitrogen; NO_3, nitrate nitrogen; pH; Temp, Temperature; TP, Total Phosphorus; circles) OTUs with Actinobacteria (blue), Cyanobacteria (red), Bacter (Bacteroidetes; gray), Proteo (Proteobacteria; gray), Firm (Firmicutes; gray) and Others (other bacteria; gray). Circle size represents OTU abundance, such as small (<2%), medium (2-5%) and large (> 5%). OTUs are grouped in major (CI and CII) and minor (Ci and Cii) consortia. List of OTUs can be found in Table S5.

Each bacterial consortium tends to be driven by some environmental variables. Consortium I shows a dominance of Cyanobacterial genera such as *Lyngbya* (Cyan-10), *Microcystis* (Cyan-12), *Prochlorococcus* (Cyan-16) and *Synechococcus* (Cyan-18) in association with members of Bacteroidetes (Bacter) and numerous minor actinobacterial OTUs; CI is driven by NO_3-N. Adjacent minor consortium Ci is dominated by *Clavibacter* (Acti-16), unclassified Actinobacteria (Acti-65) and Firmicutes (Firm); all driven by temperature. Bacteria from consortium CII are clustered around the T&O compounds MIB and geosmin (GSM); *i.e.* the dominant *Arthrobacter* (Acti-8), *Leifsonia* (Acti-34) and *Rhodococcus* (Acti-47), cyanobacteria belonging to *Anabaena* (Cyan-2), *Arthrospira* (Cyan-3), *Cyanobium* (Cyan-5), *Cyanothece* (Cyan-6), *Nostoc* (Cyan-14), *Synechocystis* (Cyan-19), *Trichodesmium* (Cyan-21). These OTUs are also grouped with Proteobacteria and other bacteria (Figure 3.5). Furthermore, the CII cluster is also linked to total phosphorus (TP; $p < 0.05$). The minor Cii consortium is represented by *Microcoleus* (Cyan-11), *Nodularia* (Cyan-13) and *Renibacterium* (Acti-46) and several minor Actinobacteria (Figure 3.5).

Bacteria and metabolic pathways

The metagenomics analysis recovered and identified several enzymes involved in the two independent metabolic pathways, *i.e.* Mevalonate (MVA) and Methyl-Erythritol-Phosphate/Deoxy-Xylulose-Phosphate (MEP/DOXP) pathways (Table 3.1). A Correspondence Analysis using enzyme reads and most abundant OTUs from each bacterial groups found in the spring sample (May 2013) when MIB and GSM concentrations were the highest in the reservoir water is illustrated on Figure 3.6. The two first axes explain more than 97.9% of the variability of the dataset, with 82.4% for axis I and 15.5% for axis II, respectively. The primary axis highlights the spatial distribution of OTUs and enzymes across the water column while the secondary axis focuses more on the sampling depth of 3 meters. It appears that the most abundant actinobacterial OTUs are gathered near the surface (00-m) and clustered around the *cmk* enzyme from the MEP whereas *Streptomyces* (Acti-58) is more present in the 3-m depth sample. Most abundant OTUs belonging to the Firmicutes looks to be exclusively present in the sub-surface sample (00-m). Cyanobacteria are stretched out between surface and 3 meters. Their abundances

tend to correlate with numerous copies of *dxs*, *dxr* and *IDI* enzyme reads. Bacteroidetes and Proteobacteria are clearly occurring the 3-m depth water layer. These two groups seem to correlate with enzymes involved in the MVA pathway. Enzymes from both pathways are more abundant in the top layers of the water column, *i.e.* 00-m and 03-m (Figure 3.6).

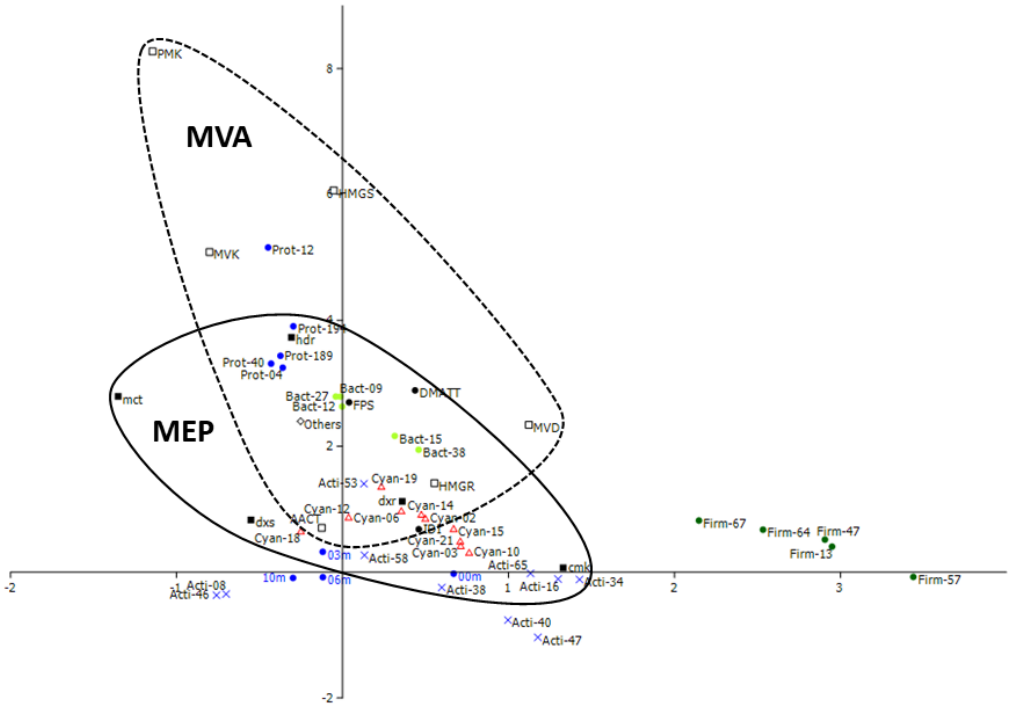

Figure 3.6: Correspondence Analysis (spring 2013) of main bacterioplankton OTUs and enzymes from the mevalonate (MVA, squares) and Methyl-Erythritol-Phosphate pathway (MEP, filled squares). Legend: Actinobacteria (blue crosses), Bacteroidetes (light green dots), Cyanobacteria (triangles), Firmicutes (dark green dots) and Proteobacteria (blue dots) and other bacteria (diamond). List of enzymes and OTUs can be found in Tables 3.1 and S5, respectively.

Table 3.1**:** Recovered enzymes from the two isoprenoid pathways MVA and MEP/ DOXP and, the two enzymes leading to precursors of monoterpenes (DMATT) and sesquiterpenes (FPS).

Pathway	Enzyme	Name	Nomenclature
Mevalonate (MVA)	AACT	acetoacetyl-CoA thiolase	EC 2.3.1.9
	HMGS	hydroxymethylglutaryl-CoA synthase	EC 2.3.3.10
	HMGR	hydroxymethylglutaryl-CoA reductase	EC 1.1.1.34
	MVK	mevalonate kinase	EC 2.7.1.36
	PMK	phosphomevalonate kinase	EC 2.7.4.2
	MVD	mevalonate 5-diphosphate decarboxylase	EC 4.1.1.33
Non-Mevalonate (MEP/DOXP)	dxs	1-deoxy-D-xylulose-5-phosphate synthase	EC 2.2.1.7
	dxr	1-deoxy-D-xylulose-5-phosphate reductoisomerase	EC 1.1.1.267
	mct	2-C-methyl-D-erythritol 4-phosphate cytidylyltransferase	EC 2.7.7.60
	cmk	4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol kinase	EC 2.7.1.148
	hdr	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	EC 1.17.1.4
	IDI	isopentenyl-diphosphate Delta-isomerase	EC 5.3.3.2
Monoterpenes	DMATT	dimethylallyltranstransferase	EC 2.5.1.1
Sesquiterpenes	FPS	farnesyl pyrophosphate synthase	EC 2.5.1.10

Discussion

Influence of nitrate nitrogen and temperature

The study of Eagle Creek Reservoir's bacterioplankton allowed us to identify four different clusters that seem to be driven by two major environmental factors, *i.e.* nitrate nitrogen and temperature (Figure 3.7). The first identified cluster CI (Figure 3.5) shows a tight connection with nitrate nitrogen and a dominance in abundance of Cyanobacteria, either benthic like *Lyngbya* (Cyan-10) or pelagic such as *Microcystis* (Cyan-12), the two picocyanobacteria *Prochlorococcus* (Cyan-16) and *Synechococcus* (Cyan-18). Next, the minor Ci cluster looks like being an offshoot of CI and tends to be more influenced by higher water temperatures which seems to be favorable for the growth of *Clavibacter* (Acti-16), Firmicutes, and many minor Actinobacteria like *Actinosynnema*, *Amycolatopsis*, *Beutenbergia*, *Cellulomonas* and *Xylanimonas*. Together, temperature and NO_3-N seem to be more favorable conditions for the growth of Actinobacteria (25 OTUs or 37.9% of total

Actinobacteria) and Firmicutes (Figure 3.7). Nitrate nitrogen is also positively correlated with pH whereas it shows a negative relationship with NH_3-N, *Arthrobacter* (Acti-8), *Geodermatophilus* (Acti-25) and *Raphidiopsis* (Cyan-17). Temperature is negatively correlated to NH_3-N and some unclassified cyanobacterial OTUs (Cyan-22). Total phosphorus is not linked to any T&O compounds although it is positively correlated to the growth of *Synechocystis* (Cyan-19; Figure 3.5).

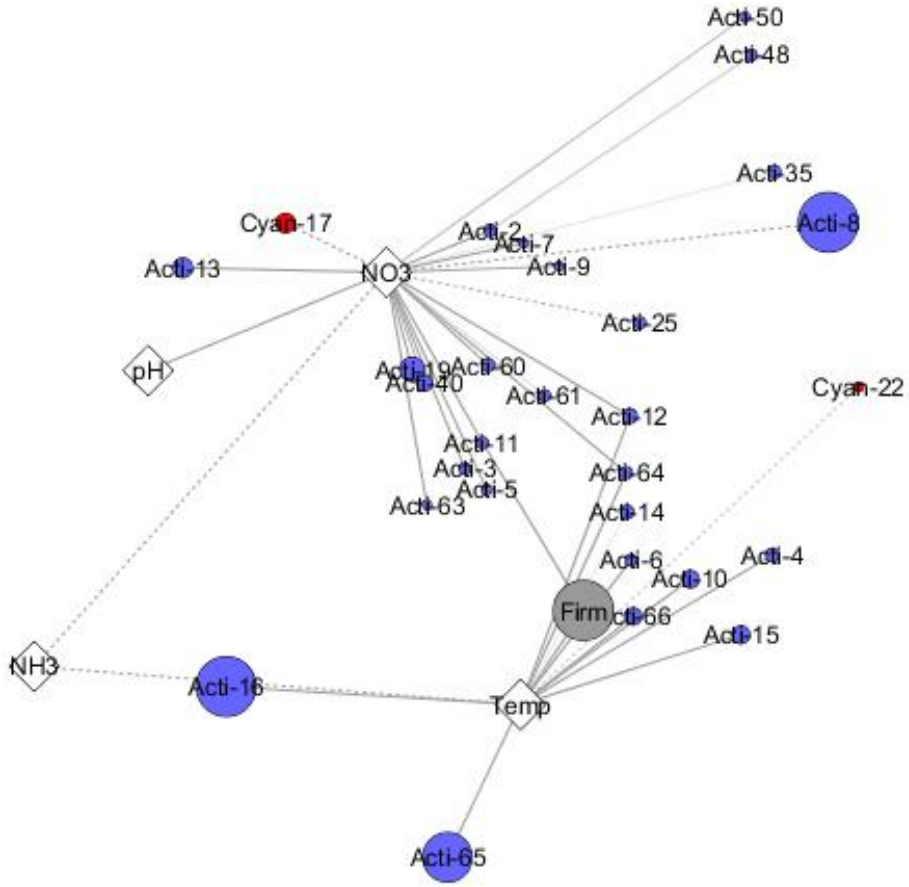

Figure 3.7: Relationships between nitrate-nitrogen, temperature and bacterioplankton OTUs in Eagle Creek Reservoir. Significant correlations ($p < 0.05$) are represented by dark colored edges and robust correlations ($p < 0.01$) by light colored edges. Circle size represents OTU abundance, as explained in Figure 3.5 legend.

Potential T&O producers

Occurrences of T&O compounds in the reservoir's waters are tightly bound to the consortium CII of bacterial OTUs (Figure 3.5). Members of Actinobacteria and Cyanobacteria are well described as strong producers of either MIB or GSM and eventually both compounds. While several OTUs are co-occurring during a taste-and-odor event, it is very often difficult to characterize individual contributions to the production of odorous compounds. Furthermore, the most abundant OTUs may not be the most active producers [*Juttner and Watson*, 2007].

Occurrences of MIB – Detections of MIB are correlated ($r > 0.6$; $p < 0.05$) to the presence of three abundant cyanobacterial OTUs (Figure 3.8A), *i.e.* the pelagic *Cyanothece* (Cyan-06) and *Synechocystis* (Cyan-19) and, the benthic *Nostoc* (Cyan-14) and the lesser abundant *Cylindrospermum* (Cyan-8). However, none of these Cyanobacteria are known producers of MIB [*Juttner and Watson*, 2007; *Watson et al.*, 2016]. Actinobacteria are well known as MIB producers; with *Streptomyces* (Actino-58) being frequently reported as a strong MIB producer [*Juttner and Watson*, 2007; *Sugiura et al.*, 1994; *Zaitlin et al.*, 2003b]. The recent discovery of the gene encoding for MIB synthase in *Micromonospora* (Acti-36) [*Citron et al.*, 2012] also broadens the spectrum of potential MIB producers in Eagle Creek Reservoir during outbreaks.

Streptomyces who are commonly found in stream, river, lake waters [*Johnston and Cross*, 1976b; *Willoughby*, 1969; *Zaitlin et al.*, 2003a] and sediments [*Jiang and Xu*, 1996] are frequently accompanied by other Actinobacteria such as *Micromonospora* and *Rhodococcus* [*Cross*, 1981]. The presence of Actinobacteria in surface waters is often found associated with sediments [*Johnston and Cross*, 1976a]. The Eagle Creek watershed characteristics, land use and soil types have not been investigated during this study but Actinobacteria found in the reservoir waters are possibly coming from the agricultural soil erosion. Spore-carrying soil particles would be transported by Eagle Creek and other tributaries to finally seed the reservoir.

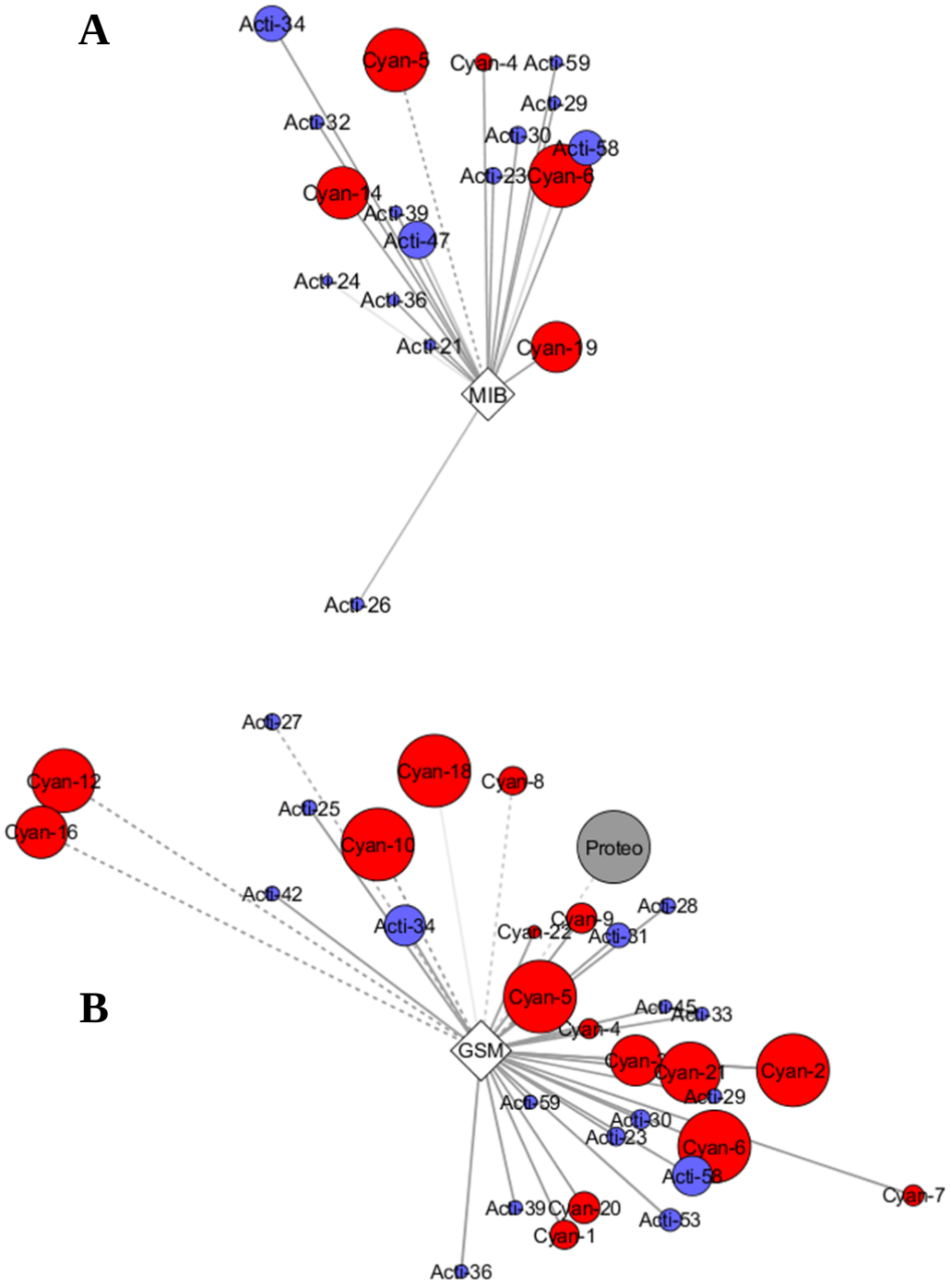

Figure 3.8: Relationships between bacterioplankton OTUs and A) MIB and, B) Geosmin (GSM). Significant correlations ($p < 0.05$) are represented by dark colored edges and robust correlations ($p < 0.01$) by light colored edges. Circle size represents OTU abundance, as explained in Figure 3.5 legend.

Occurrences of GSM – On Figure 3.8B, there is a strong correlation ($r > 0.7$; $p < 0.01$) between GSM detections and occurrences of *Synechococcus* (Cyan-18) although this OTU is not known as a potential producer [*Juttner and Watson*, 2007]. Moreover, seven other co-occurring cyanobacterial OTUs positively correlate as well ($p < 0.05$) but only two are well-known producer of geosmin; *i.e. Anabaena* (Cyan-02) [*Tsao et al.*, 2014] and *Trichodesmium*. However, an anomaly was detected here as *Trichodesmium* is almost exclusively described as a marine organism [*Capone et al.*, 1997]. Routine visual identification of cyanobacterial taxa under a photonic microscope reported co-occurrences of *Planktothrix agardhii* and *P. rubescens* in Eagle Creek Reservoir during the month of May 2013. This genus is frequently cited as a MIB- and geosmin-producing bacteria [*Durrer et al.*, 1999; *Juttner and Watson*, 2007]. In addition, *Trichodesmium* and *Planktothrix* belong to the same new family rank (*status novus*) *Microcoleaceae* (ex-*Phormidiaceae*) from the cyanobacterial order of Oscillatoriales [*Komárek et al.*, 2014] and a misidentification in the *Refseq* database could have easily happened due to the phylogenetic proximity/similarity of these two OTUs (Supplemental Table S6).

The geosmin-producing *Streptomyces* (Acti-58) [*Juttner*, 2007], non-producing *Leifsonia* (Acti-34) and 13 other less abundant actinobacterial OTUs also show a positive relationship with GSM. Among these lesser abundant OTUs, presumed geosmin synthases were identified in *Frankia* (Acti-23) and *Saccharopolyspora* [*Ghimire et al.*, 2008], cryptic and silent synthase genes have been revealed in others like *Streptosporangium* (Acti-59) [*Yamada et al.*, 2012] and recently confirmed in *Nocardiopsis* (Acti-42) [*Sun et al.*, 2017]. Again, individual contributions to the final signal of geosmin recorded in Eagle Creek Reservoir is hard to assess and the origin of GSM outbreaks may come from a cocktail of various OTUs belonging to both Cyanobacteria and Actinobacteria.

Metabolic pathways and enzymes

In May 2013, during the major episode of T&O, the upper layers (0-3 meters) of the water column sheltered most of the Actinobacteria and Cyanobacteria OTUs (Figure 3.6). Elevated densities of potential producers in these layers co-occurred with high abundances of both MVA and MEP enzymes which were undoubtedly active, compared to the deepest layers of 6 and 10 meters (Figure 3.6). As the *SEED Subsystems* database from

the MG-RAST server was unable to directly recover the key enzymes of interest; *i.e.* methylisoborneol synthase (EC 4.2.3.118) and germacradienol-geosmin synthase (EC 4.1.99.16), we focused our attention on the two upstream enzymes producing the immediate compounds utilized by MIB and geosmin synthases. Thus, dimethyl-allyl-trans-transferase (DMATT; EC 2.5.1.1), that catalyzes the reaction between dimethyl-allyl pyrophosphate and isopentenyl pyrophosphate (IPP), forms geranyl pyrophosphate (GPP) which leads to MIB production [*Giglio et al.*, 2011; *Komatsu et al.*, 2008] and, farnesyl pyrophosphate synthase (FPS; EC 2.5.1.10) catalyzes the conversion of GPP and IPP into farnesyl pyrophosphate (FPP) towards geosmin production [*Cane et al.*, 2006; *Jiang et al.*, 2007]. On Figure 3.6, DMATT is abundant at the depths of 0 and 3-m whereas FPS is abundantly found at 3 meters. This difference in the spatial distribution seems to match the distribution of the two major groups of potential producers. DMATT near the surface mimics the presence of the most abundant Actinobacteria while FPS follows the pattern of Cyanobacteria. *Anabaena* (Cyan-02), *Trichodesmium/Planktothrix* (Cyan-21) and *Streptotmyces* (Acti-58) previously identified as the major players in the production of MIB and GSM in Eagle Creek Reservoir are also found clustered near the isopentenyl-diphosphate delta-isomerase (IDI; EC 5.3.3.2) which is responsible of the reversible conversion of two isoprenoid precursors: isopentenyl pyrophosphate (IPP) and dimethyl-allyl pyrophosphate (DMAPP), independently synthesized by the MVA or MEP pathways [*Lombard and Moreira*, 2010]. This close connection demonstrates that both pathways and enzymes are active in the upper layers, although the MEP pathway is the preferred route of Actino- and Cyanobacteria [*Kuzuyama*, 2002; *Lange et al.*, 2000]. The GPP monoterpene precursor can also lead to the synthesis of other odorous compounds such as limonene, pinene or camphor [*Trudgill*, 1990] and, FPP the product of FPS is the precursor to a wide variety of sesquiterpenes, aromas and essential oils, such as germacrene [*Dewick*, 2002] and its cometabolite germacradienol from whom geosmin would be a degradation by-product [*Yamada et al.*, 2015]. Nevertheless, the detection of MIB and GSM compounds in Eagle Creek waters means that both enzymes, DMATT and FPS, likely were active in the synthesis of T&O compounds.

Fate of MIB and Geosmin

Since Actinobacteria and Cyanobacteria are abundant in natural water ecosystems, it is expected that the microbial breakdown of T&O compounds occurs. MIB has been found to be degraded by *Pseudomonas*, *Enterobacter* [*Izaguirre et al.*, 1988; *Tanaka et al.*, 1996], *Flavobacterium* [*Egashira et al.*, 1992] and *Bacillus* [*Ishida and Miyaji*, 1992; *Lauderdale et al.*, 2004] while geosmin is degraded by *Rhodococcus* and *Arthrobacter* [*Saadoun and El-Migdadi*, 1998], *Sphingopyxis* [*Hoefel et al.*, 2009], *Sphingomonas* and *Novosphingobium* [*Ho et al.*, 2007] and, *Comamonas* [*Guttman and van Rijn*, 2012]. A slow degradation of T&O compounds can be stimulated by addition of ethanol which activates alcohol dehydrogenase [*Saito et al.*, 1999]. This enzyme could enhance the degradation of MIB and geosmin as both are tertiary alcohols. Our data support that microbial degradation can occur in Eagle Creek Reservoir because of the presence of *Flavobacterium*, *Novosphingobium*, *Sphingomonas* and *Pseudomonas*. This would require a high microbial degradation rate to effectively remove elevated concentrations of MIB and geosmin in water bodies with long residence times, such as drinking water supply reservoirs and fish ponds. Unfortunately, information about microbial degradation rates of MIB or GSM in natural environments is not available in the current literature. Furthermore, several authors reported that both MIB and GSM compounds can simultaneously be found as dissolved or as cell-/particle-bound [*Durrer et al.*, 1999; *Jähnichen et al.*, 2011; *Juttner and Watson*, 2007; *Peter et al.*, 2009]. The fractionation of these compounds in the water would affect their availability for biodegradation, their steadiness and even the efficiency of removal rates during the coagulation, flocculation and clarification in drinking water treatment plants.

Conclusions

The present study was able to characterize and identify bacterial OTUs in a eutrophic reservoir during an outbreak of MIB and geosmin as well as relationships between these organisms. Two major consortia of bacterioplankton were described. A consortium CI with a dominance of Cyanobacteria was driven by temperature, nitrate nitrogen and was reflective of epilimnetic conditions before nutrient exhaustion during the

summer stratification. The consortium CII mostly described conditions during T&O outbreaks with high concentrations of MIB and GSM in May, and in a lesser extent in October when water columns were partially mixed. A co-dominance of Actinobacteria and Cyanobacteria was observed in CII who were almost exclusively found in the upper layers of the water column, between 0 and 3 meters. Potential producers of MIB in Eagle Creek Reservoir have been identified as the actinobacterial *Streptomyces* and *Micromonospora* whereas geosmin-producing OTUs were the cyanobacterial *Anabaena* and *Trichodesmium/ Planktothrix* and, eventually *Streptomyces*. Individual contribution to the global signal of each T&O compound is difficult to assess. Delays and shifts in the growth of co-occurring species may also blur the signal from minor contributors. Despite the inability to recover key enzyme reads from MIB and GSM synthases, immediate upstream enzymes (DMATT and FPS) were also found abundant in the upper layers of the water column. Their presence highlights an active biosynthesis of mono- and sesquiterpenes associated with high abundances of Actino- and Cyanobacteria.

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CHAPTER 4 – OCCURRENCES OF (MIB, GEOSMIN) – DEGRADING BACTERIA IN A EUTROPHIC RESERVOIR AND THE ROLE OF CELL-BOUND VERSUS DISSOLVED FRACTIONS

'Don't forget that the flavors of wine and cheese depend upon the types of infecting microorganisms'
Martin H. Fischer

Introduction

World major cities rely on surface waters as an important source for drinking water. Raw waters must undergo a treatment process throughout a multi-step procedure before human consumption. Generally, basic treatment processes consist of particle separation, oxidation and adsorption to meet quality, aesthetics and microbiological requirements. Failures in fulfilling these requirements may lead to customers' negative perception of drinking water quality and confront water utilities to customer complaints and a loss of trust [*Suffet et al.*, 1996]. Taste-and-Odor (T&O) compounds like 2-methylisoborneol (MIB) and geosmin (GSM) often compromise the aesthetic properties of drinking waters. These tertiary alcohols have relatively low molecular weights; *i.e.* 168.28 g. mol^{-1} for MIB and 182.31 g. mol^{-1} for GSM [*Pirbazari et al.*, 1992], moderate solubility, and moderate hydrophobicity [*Song and O'Shea*, 2007]. Reported odor threshold values are very low, thus concentrations as low as 4 ng. L^{-1} (or parts per trillion) for GSM and 15 ng. L^{-1} for MIB can readily be detected by human olfactory sense [*Peter et al.*, 2009; *Young et al.*, 1996].

In source waters, the production of T&O compounds is caused by microorganisms [*Juttner and Watson*, 2007] belonging to numerous Actinobacteria and seasonal bloom-forming Cyanobacteria [*Juttner and Watson*, 2007; *Li et al.*, 2007; *Su et al.*, 2015; *Watson et al.*, 2008]. Technically, T&O issues can be handled in two different ways; either controlled at the source or in the water treatment plant. First, reducing nutrient loads into water bodies may achieve long-term management goals in an effort of limiting algal or bacterial growth [*Paerl et al.*, 2011]. Punctual applications of copper-based algaecides

provide the same results on the short term [*Murray-Gulde et al.,* 2002] although small areas of a lake/reservoir can only be treated due to associated elevated cost. In addition, there are known negative side effects to using copper in aquatic environments as it negatively affects autotrophic bacteria [*Le Jeune et al.*, 2007], invertebrates [*Joachim et al.*, 2017] and fish [*Song et al.*, 2015]. Copper also accumulates in sediments where potential changes in the water chemistry may later release it and become a long-term problem [*Haughey et al.*, 2000]. Secondly, an enhancement of T&O removal in a drinking water treatment plant can be achieved if seasonal fluctuations are anticipated by monitoring the dynamics of potential producers [*Westerhoff et al.*, 2005]. Therefore, the screening of T&O compounds in surface waters is utterly important for managers who want to curb forthcoming odorous outbreaks by adapting their treatment dosages for efficient removal [*Bruce et al.*, 2002]. The chemical control of microbial growth is often limited by the risks of disrupting cell walls and thus releasing intracellular T&O compounds. Releases of intracellular MIB and Geosmin exceeding Odor Threshold Concentrations (OTC) were observed from a few cyanobacterial genera such as *Oscillatoria* and *Lyngbya* after treatment with chlorine, chlorine dioxide and chloramine [*Wert et al.,* 2014]. Different techniques of water treatment and purification processes have been optimized as cell-bound fractions of MIB or geosmin do not behave like their dissolved counterparts [*Srinivasan and Sorial*, 2011]. The physical removal of cell-bound or intracellular T&O is preferred and cell disruption must be avoided as it releases other dissolved organics exacerbating the problem [*Peterson et al.*, 1995]. The chemical adsorption of dissolved compounds requires the use of activated carbons [*Durrer et al.*, 1999] but is challenged by the presence of dissolved organic carbon in water which competes for adsorption sites [*Bruce et al.*, 2002]; with geosmin showing greater removal rates than MIB on activated carbons [*Zamyadi et al.*, 2015]. Effective removals of the recalcitrant dissolved MIB is usually accomplished using ozonation [*Liang et al.*, 2006] as the formation of hydroxyl radicals ($HO^{\bullet}$) degrade the molecule [*Westerhoff et al.*, 2006] primarily into *d*-camphor [*Qi et al.*, 2009].

Biological methods for the removal of MIB and geosmin have received little attention in the drinking water industry as applications are limited to bio-filtration through sand filters [*Srinivasan and Sorial,* 2011]. The first study conducted on the biological removal of geosmin demonstrated that bacterial degradation was ineffective in drinking

water [*Huck et al.*, 1995]. Since, other investigations have shown the contrary. Biodegradation of geosmin was identified in a consortium of three bacteria (*Sphingopyxis alaskensis, Novosphingobium stygiae* and *Pseudomonas veronii*) isolated from a sand filter biofilm [*Hoefel et al.*, 2006]. Subsequently, the removal of both MIB and GSM was established in biologically active sand filters by four bacteria identified as an *α-Proteobacterium*, an *Acidobacteriaceae*, *Pseudomonas sp.*, and *Sphingomonas sp.* [*Ho et al.*, 2007]. Over the past decade, most studies conducted on bio-filtration have used sand or activated carbon [*Doederer et al.*, 2018; *Elhadi et al.*, 2006; *Metz et al.*, 2006; *Persson et al.*, 2007; *Zamyadi et al.*, 2015]. To date, only a few investigations tried to identify potential degraders from natural environments, such as rivers [*Du et al.*, 2017], reservoirs [*Westerhoff et al.*, 2005] and drinking water supplies [*Ho et al.*, 2012]. Currently, the knowledge of bacteria capable of degrading T&O compounds is still sparse.

In Eagle Creek Reservoir, a eutrophic water body providing drinking water to the city of Indianapolis, seasonal episodes of either MIB, geosmin or both compounds have been recorded for almost two decades by the local water company. Sources of T&O outbreaks were elucidated and members of Actinobacteria and Cyanobacteria were identified to correlate to T&O occurrences (*cf.* Chapter 3). The objectives of the present study are to identify which naturally occurring bacterial degraders were dominant during the two 2013 T&O outbreaks and then, to determine which ones of these Operational Taxonomic Units (OTUs) had the capability to degrade T&O compounds. Furthermore, the fractionation of cell-bound versus dissolved compounds will also be investigated to highlight different chemical behaviors and their influence on the dynamics of bacterial degraders.

Materials and Methods

Study site

Eagle Creek Reservoir, located in central Indiana (Figure 4.1), receives drainage from the Eagle Creek Watershed (419.6 km^2; HUC 05120201120) and has a surface area of 5.7 km^2. Since 1967, the reservoir has been providing drinking water for the city of Indianapolis and surrounding communities. The maximum depth ranges from about 11 to

13 meters, with the deepest areas located in the southern basin, near the dam. Eagle Creek Reservoir is a small, dimictic, and eutrophic water body with seasonal thermal stratification from June to September. Reservoir mixings usually occur in April/May and October each year.

Figure 4.1: Eagle Creek Reservoir sampling site (gray dot), south of West 56th street.

Sample collection and processing

Water collection – Water samples were collected from May to October 2013 on a biweekly basis near the reservoir water intake located south of W. 56th street (Figure 4.1). Epilimnetic water samples were collected with a vertical Van Dorn sampler at the depth of 3 meters. A total of 11 samples were collected throughout the sampling campaign covering the summer stratification and the spring and fall mixing periods. A submersible multi-parameter V2-6600 YSI probe (YSI, Inc., Yellow Spring, OH) was deployed each time to measure water temperature (Temp, °C) and pH (*s. u.*) at the given depths.

T&O compounds analysis – Water sample splits were stored in brown amber glass jars with no headspace or bubbles to avoid the volatilization of methylisoborneol and geosmin. All samples were stored on ice for transport to the laboratory. T&O compound

fractionation was realized using 0.22 µm syringe-mounted filters and then, concentrations were quantified by Head-Space Solid-Phase Micro-Extraction (HS-SPME) combined with a Gas Chromatography-Mass Spectrometry (GC-MS) [*APHA*, 2000].

Bacterioplankton community composition – After collection, all water samples were put on ice in autoclaved 1-L HDPE brown bottles and filtered in the lab through 0.22 µm mesh size pores on a sterile glass filtration manifold. Filters were then frozen for storage in 15-mL Falcon tubes. Samples were later shipped to Illumina, Inc., San Diego, CA for analysis on frozen filters to determine the phylogenetic structure of the BCC by using a 460 bp-long amplicon amplified by polymerase chain reaction (PCR). The gene-specific sequences target the 16S rRNA V3 and V4 regions (MiSeq v.3 Nextera XT, Illumina): 5'TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGG CWGCAG and, 5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACH VGGGTATCTAATCC as forward and reverse primers respectively.

Statistical Analysis

Descriptive statistics were run on the 16S and T&O datasets using PAST 3.1 software [*Hammer et al.*, 2001]. Spearman's *rho* (r_s) correlations were used to highlight the robustness of relationships between occurrences of bacterial degraders and T&O concentrations in the reservoir water. In this study, only highly significant relationships; *i.e.* $r_s > 0.73$ ($p < 0.01$) and $r_s > 0.83$ ($p < 0.001$) were retained in order to identify with confidence potential degraders.

Results

Seasonal fractionation of Geosmin and MIB

Between May and November 2013, the reservoir raw water was analyzed intensively (n= 134) in order to illustrate the fractionation of MIB and geosmin. Results of total concentrations and fractionations (cell-bound versus dissolved) are shown on Figure 4.2. Three major phases were identified: phases I and III as odorous outbreaks when total concentrations of either MIB or GSM exceeded respective OTCs; *i.e.* 15 ng L^{-1} (MIB) and 4 ng L^{-1} (GSM) [*Peter et al.*, 2009] and, phase II as non-odorous. Throughout the sampling

season, MIB was mostly found dissolved in water while GSM was more frequently cell-bound. MIB barely exceeded its OTC (14.2%) whereas GSM overpassed it 76.9% of the times (Table 4.1).

Phase I – From May 1st and June 15th, MIB and GSM co-occurred during the spring outbreak. Year maxima in total concentrations were reached during phase I with 99.1 and 77.3 ng L^{-1} of MIB and geosmin, respectively. Both compounds exceeded their respective OTC more than 50% of the times across Phase I (Table 4.1). Most of the MIB was found in the dissolved fraction while GSM seemed to be mainly cell-bound (Figure 4.2), with respective bound vs. dissolved (B:D) ratios of 0.21 and 0.97 respectively (Table 4.1).

Phase II – During this phase, from June 16th to August 26th, total concentrations of MIB and GSM in water were extremely low and did not exceed 6 ng L^{-1} and only GSM reached its OTC (Figure 4.2). Year minima for MIB and GSM concentrations were recorded during phase II as both compounds were mostly found in the dissolved fraction, resulting in low observed B:D ratios. This phase was described as non-odorous as MIB always remained lower than its OTC value and GSM only attained it 38.8% of the times (Table 4.1).

Phase III – The second odorous outbreak began on August 27th and extended beyond the end of sampling campaign. Moderate concentrations were recorded in the reservoir water with averages of 8.3 and 9.4 ng L^{-1} of MIB and geosmin, respectively. However, MIB never exceeded its OTC whereas GSM was always above with a peak concentration of 20.1 ng L^{-1}. Similarly to phase I, MIB was almost exclusively dissolved with its B:D ratio of 0.04 while GSM was dominantly cell-bound with a B:D ratio that bounced back to 0.80 (Table 4.1).

Table 4.1: MIB and geosmin concentrations, fractionations expressed as Bound: Dissolved (B:D) ratios, during the whole 2013 campaign and major odorous outbreaks I, II and III. OTC: Odor Threshold Concentration.

	2013		Phases					
	Campaign		I		II		III	
Compound	**MIB**	**GSM**	**MIB**	**GSM**	**MIB**	**GSM**	**MIB**	**GSM**
n	134	134	37	37	49	49	48	48
min (ng L^{-1})	1.0	2.1	5.4	3.9	2.0	2.1	2.1	4.0
max (ng L^{-1})	99.1	77.3	99.1	77.3	7.5	6.0	13.4	20.1
mean (ng L^{-1})	14.1	11.8	35.5	25.8	3.7	3.7	8.3	9.4
OTC Exceedance								
n>OTC	19	103	19	36	0	19	0	48
%>OTC	14.2	76.9	51.4	97.3	0.0	38.8	0.0	100.0
Fractionation								
mean % bound (B)	9.1	38.0	17.0	49.3	92.1	76.7	4.1	44.3
mean % dissolved (D)	90.9	62.0	83.0	50.7	7.9	23.3	95.9	55.7
B:D ratio	0.10	0.61	0.21	0.97	0.09	0.30	0.04	0.80

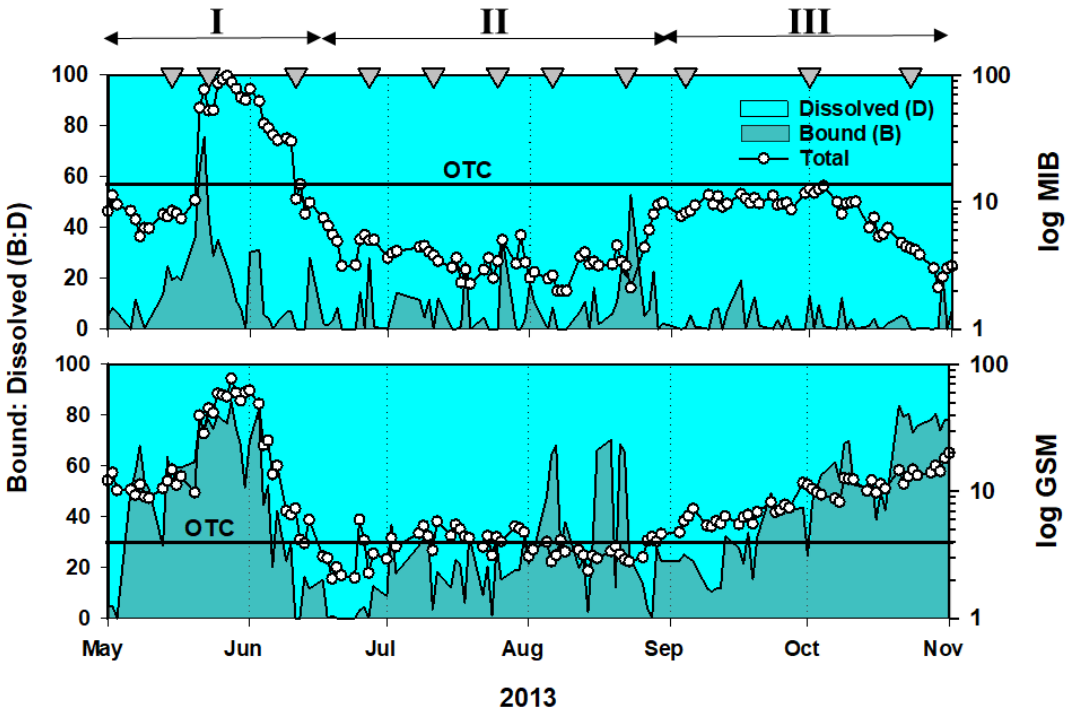

Figure 4.2: Fractionation of MIB (top panel) and GSM (bottom panel) in 2013 Eagle Creek Reservoir raw water. Legend: dissolved fraction (light gray); particle-bound fraction (dark gray); total concentration (dotted line, in ng L^{-1} or ppt). OTC: Odor Threshold Concentration = 15 ng L^{-1} (MIB) and 4 ng L^{-1} (GSM). Odorous outbreaks are marked as phases exceeding the respective OTC and labelled I, II and III. Reverse triangles indicate sampling dates.

Phasic dynamics of T&O-degrading bacteria

In Eagle Creek Reservoir, the whole bacterioplankton community composition was overall driven by the presence of four major phyla. Based on the 16S rRNA analysis, results showed that Cyanobacteria were the most abundant group with an average of 42.8% of the reservoir's microbiome, followed by Proteobacteria (21.8%), Bacteroidetes (6.5%) and Actinobacteria (3.7%) (Figure 4.3). Interestingly, Cyanobacteria and Actinobacteria include known producers of MIB and geosmin [*Juttner and Watson*, 2007; *Watson et al.*, 2016] while some members of Proteobacteria, Bacteroidetes, Firmicutes and also a few Actinobacteria are representative of potential degraders of these compounds (Table 4.2). Throughout the 2013 sampling campaign, a total of 138 OTUs potentially capable to degrade either MIB (n=96) or GSM (n=42) were identified. MIB-degraders tended to be grouped within Bacteroidetes (Bact), Firmicutes (Firm) and γ-Proteobacteria (γ-Prot) whereas GSM-degrading OTUs belonged to α-Proteobacteria (α-Prot), β-Proteobacteria (β-Prot), and Actinobacteria (Acti) groups (Appendix I).

Phase I – During the main T&O event, Proteobacteria were the most abundant with 31.8% of total bacterial abundance (Figure 4.3): 35 OTUs of almost exclusively *Pseudomonas spp.* (γ-Prot), 27 OTUs of *Novosphingobium spp.*, *Sphingomonas spp.* and *Sphingopyxis spp.* (α-Prot), and 5 OTUs of *Comamonas spp.* (β-Prot) (Table 4.3). Other co-occurring OTUs belonged to *Flavobacterium spp.* (Bact). All of these phyla were found dominant during Phase I (Figure 4.4).

Phase II – A dominance of cyanobacterial OTUs (>50%) were observed during phase II which matched to the summer period. All other major phyla showed a significant drop compared to phase I (Fig. 4.3). The B:D ratio of geosmin remained around 0.30 (Table 4.1), and several GSM-degraders were found in the reservoir water, including: *Novosphingobium spp.* (α-Prot; Figure 4.4a) and *Rhodococcus spp.* (Acti; Figure 4.4d) whose abundances increased, contrary to *Sphingomonas ssp.* (Figure 4.4b) and *Comamonas spp.* (Figure 4.4c) whose abundances decreased. On the other hand, the abundances of potential MIB-degraders such as *Pseudomonas spp.* (Figure 4.4e) and *Flavobacterium spp.* (Figure 4.4f) were minimal. *Enterobacter spp.* (Figure 4.4g) who were not so abundant during Phase I almost disappeared as the B:D ratio of MIB dropped to 0.09 (Table 4.1).

Phase III – The second odorous event of the reservoir showed a dominance of Cyanobacteria (47.1% of total microbiome) and an increase in Proteobacteria (19.9%) and Firmicutes (4.7%) abundances. The rise of cell-bound geosmin in water; *i.e.* elevated B:D ratio of 0.80 (Table 4.1) seemed to be beneficial to support the growth of some *Novosphingobium* and *Sphingomonas* species, both α-Proteobacteria (Figure 4.3). Although not known as a potential degraders of GSM, Firmicutes represented by *Bacillus spp.* increased significantly with this increase of cell-bound geosmin while all other MIB-degrading OTUs (Figures 4.4e, f and g) were almost gone due to the scarcity of MIB (<13.4 ng L^{-1}; Table 4.1).

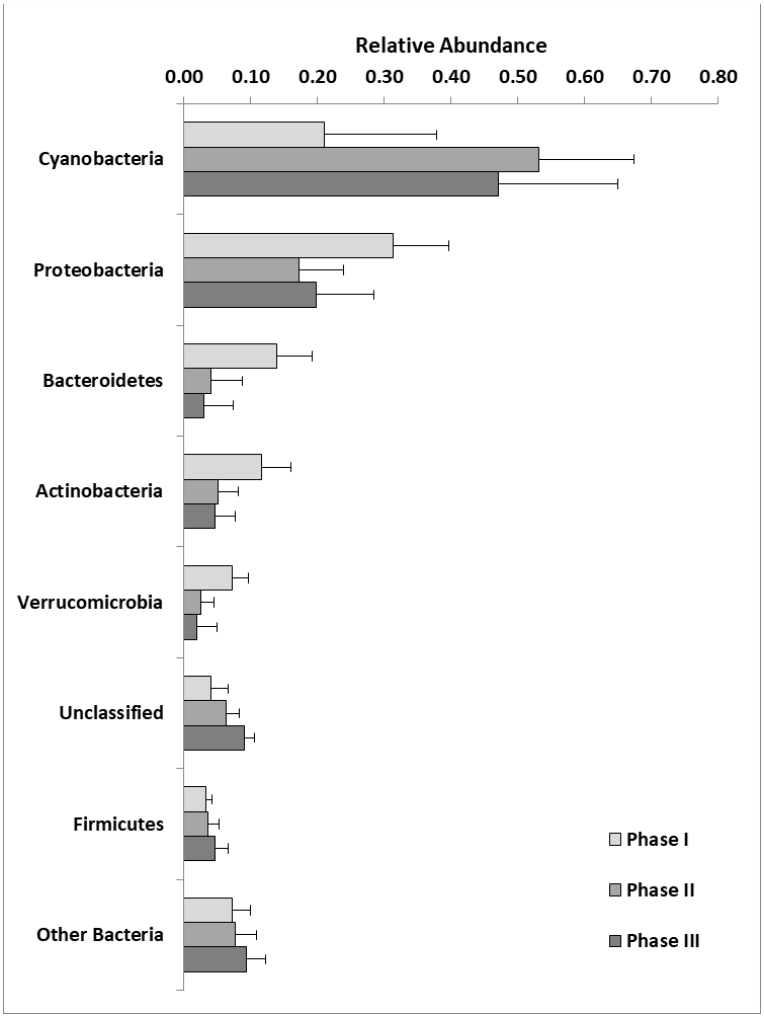

Figure 4.3: Relative abundances of major bacterioplankton phyla during the three phases of the reservoir. Bars represent standard deviations.

Table 4.2: Known MIB and geosmin (GSM)-degrading bacteria.

Actinobacteria	**Compound**	**Source**
Arthrobacter atrocyaneus	GSM	[*Saadoun and El-Migdadi*, 1998]
Arthrobacter globiformis	GSM	[*Saadoun and El-Migdadi*, 1998]
Brevibacterium spp.	MIB	[*Yuan et al.*, 2012]
Chlorophenolicus strain N-1053	GSM	[*Saadoun and El-Migdadi*, 1998]
Micrococcus spp.	MIB	[*Yuan et al.*, 2012]
Rhodococcus moris	GSM	[*Saadoun and El-Migdadi*, 1998]
Rhodococcus ruber T1	MIB	[*Eaton and Sandusky*, 2009]
Rhodococcus wratislaviensis DLC-cam	MIB, GSM	[*Eaton and Sandusky*, 2010]
Bacteroidetes		
Chryseobacterium sp.	GSM	[*Zhou et al.*, 2011]
Flavobacterium multivorum	MIB	[*Egashira et al.*, 1992]
Flavobacterium spp.	MIB	[*Egashira et al.*, 1992; *Yuan et al.*, 2012]
Firmicutes		
Bacillus cereus	GSM	[*Narayan and Nunez III*, 1974; *Silvey et al.*, 1970]
Bacillus subtilis	MIB, GSM	[*Narayan and Nunez III*, 1974; *Yagi et al.*, 1988]
Bacillus idriensis	MIB	[*Du et al.*, 2017]
Bacillus spp.	MIB	[*Ishida and Miyaji*, 1992; *Lauderdale et al.*, 2004]
α-Proteobacteria		
Novosphingobium stygiae	GSM	[*Hoefel et al.*, 2006]
Sinorhizobium sp.	GSM	[*Zhou et al.*, 2011]
Shinella zoogloeoides	MIB	[*Du et al.*, 2017]
Sphingomonas sp.	GSM	[*Ho et al.*, 2007]
Sphingopyxis alaskensis	GSM	[*Hoefel et al.*, 2006]
Sphingopyxis sp. Geo48	GSM	[*Hoefel et al.*, 2009]
β-Proteobacteria		
Comamonas sp.	GSM	[*Guttman and van Rijn*, 2012]
γ-Proteobacteria		
Enterobacter spp.	MIB	[*Tanaka et al.*, 1996]
Pseudomonas aeruginosa	MIB	[*Egashira et al.*, 1992]
Pseudomonas putida G1	MIB	[*Eaton and Sandusky*, 2009; *Oikawa et al.*, 1995]
Pseudomonas spp.	MIB, GSM	[*Egashira et al.*, 1992; *Ho et al.*, 2007; *Izaguirre et al.*, 1988; *Tanaka et al.*, 1996; *Yuan et al.*, 2012]
Pseudomonas sp. SBR3-tpnb	GSM	[*Eaton and Sandusky*, 2010]
Pseudomonas veronii	GSM	[*Hoefel et al.*, 2006]
Stenotrophomonas sp.	GSM	[*Zhou et al.*, 2011]

Table 4.3: Phasic occurrences of T&O-degrading OTUs.

	n	**Phase I**	**Phase II**	**Phase III**
Actinobacteria	4	4 (100%)	1 (25.0%)	1 (25.0%)
Bacteroidetes	31	30 (96.8%)	22 (71.0%)	19 (61.3%)
Firmicutes	24	12 (50.0%)	9 (37.5%)	18 (75.0%)
α-Proteobacteria	34	27 (34.2%)	25 (31.6%)	21 (26.6%)
β-Proteobacteria	6	5 (6.3%)	3 (3.8%)	3 (3.8%)
γ-Proteobacteria	41	35 (44.3%)	13 (16.5%)	9 (11.4%)

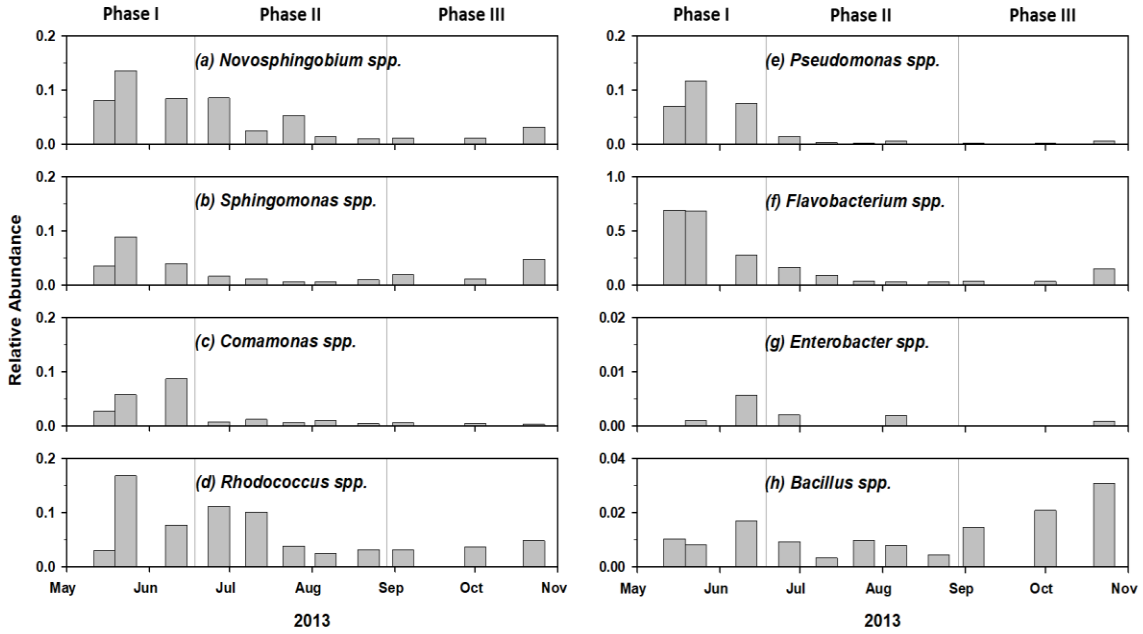

Figure 4.4: Phasic dynamics of main potential degraders of geosmin (a, b, c, d) and MIB (e, f, g, h). Relative abundances are expressed as percent of total microbiome.

Identification of potential T&O-degrading bacteria

Out of the 138 identified OTUs with the potential capability to degrade T&O compounds, only a small part showed a significant positive correlation with either MIB or GSM concentrations and/or B:D ratios (Appendix II). The identification of potential degraders was assessed, using Spearman's *rho*, according to best correlation factors and levels of significance between co-occurrences of a given OTU and concentrations of T&O compounds in the water.

MIB-degrading bacteria – Amongst the 29 identified potential degraders of MIB, 12 OTUs belonged to the genus *Flavobacterium* (Bacteroidetes); with *F. resistens, F. granuli, F. saliperosum* (p <0.001), *F. kamogawaensis* (p <0.01) and the remaining 8 OTUs with p <0.05 (Figure 4.5A). Eight *Pseudomonas* species (γ-Prot) only showed a p value of 0.05, with *Pseudomonas teessidea* having the highest correlation factor. Then, *Bacillus soli* (p <0.05) was the only representative of the Firmicutes. A total of 8 other OTUs belonging to phyla not known as capable to degrade MIB compounds did show a strong relationship: *Comamonas odontotermitis* (β-Prot; p <0.001), and seven α-Proteobacteria: *Sphingomonas*

oligophenolica (p <0.001), *S. hunanensis, S. soli, S. panipatensis* (p <0.05), *Novosphingobium subterraneum* (p <0.01), *N. lentum and N. hassiacum* (p <0.05).

GSM-degrading bacteria – A total of 17 OTUs showed a positive and significant correlation with geosmin concentrations in the water but only three of them belonged to phyla of known degraders: the two α-Proteobacteria *Novosphingobium hassiacum* (p <0.001), *Sphingomonas oligophenolica* (p <0.01) and, *Comamonas odontotermitis* (β-Prot; p <0.05). Amongst the 9 *Flavobacterium* species, only *F. saliperosum* had the strongest relationship with geosmin (p <0.01). *Bacillus niacini* (Firmicutes) and the four *Pseudomonas* species (γ-Prot): *P. umsongensis, P. teessidea, P. corrugata and P. lundensis* were statistically significant (p <0.05) (Figure 4.5B).

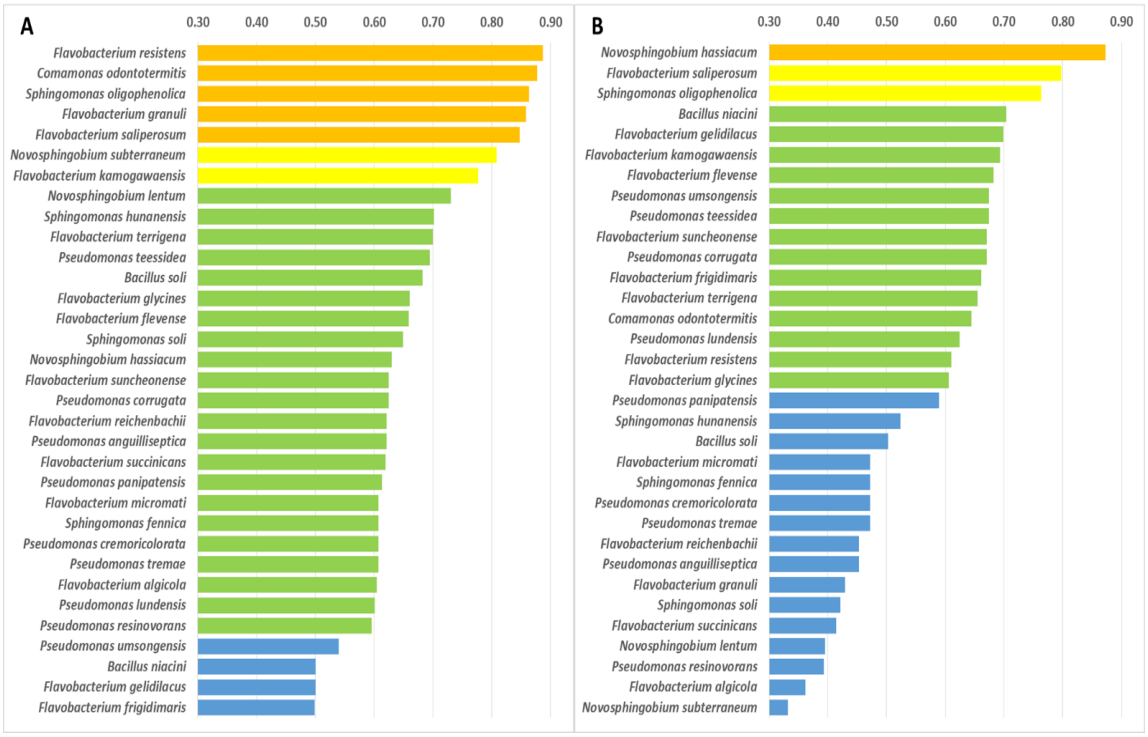

Figure 4.5: List of the 33 bacterial OTUs showing a positive correlation (Spearman's *rho*; x-axis) with A) methylisoborneol and, B) geosmin. Level of significance is *rho* > 0.60 and displayed using colored bars as follows: blue (non-significant), green (p < 0.05), yellow (p < 0.01) and orange (p < 0.001).

The case of Pseudomonas – As previously mentioned, many OTUs belonging to *Pseudomonas* have an undetermined role in T&O compounds biodegradation. They mainly occurred during phase I (Figure 4.4) when both T&O compounds were elevated. Most of them had *p* values lower than 0.05 for either MIB or GSM compounds (Figure 4.5). These weaker correlations can be explained by the ambivalence of the *Pseudomonas* group with some OTUs described as MIB and/or GSM degraders (Table 4.2). Although the respective role and the individual contribution of each *Pseudomonas* species cannot be explained with confidence from our dataset, they may or not have played a role in the biodegradation of T&O compounds in Eagle Creek Reservoir and, any correlation to MIB or GSM could simply be coincidental and not casual.

Discussion

T&O degraders and odorous compounds

The dynamics of T&O-degrading bacteria in Eagle Creek Reservoir mimicked the seasonal peaks of MIB and geosmin. These bacteria were more abundant during phases I and III, showed lower abundances and, for some, fewer occurrences during phase II (Figure 4.4). Odorous episodes with elevated concentrations of T&O compounds in the reservoir's water supported the growth of numerous co-occurring OTUs. The most abundant MIB-degrading bacteria were Bacteroidetes represented by several species of *Flavobacterium* as described in some earlier studies [*Egashira et al.*, 1992; *Yuan et al.*, 2012]. In this study, we were able to identify four *Flavobacterium* species (*F. resistens, F. granuli, F. saliperosum, F. kamogawaensis*) with the potential capability to degrade MIB (Figure 4.5A). Several *Pseudomonas* species mainly occurred during phase I while MIB concentrations were the most elevated. Despite their low significance ($p < 0.05$), highest abundances of *P. teessidae, P. corrugata, P. anguilliseptica, P. panipatensis, P. cremoricolorata, P. tremae, P. lundensis* and *P. resinovorans* were found in phase I (Appendix II). This suggests that *Pseudomonas spp.* could have used MIB as a carbon source [*Eaton and Sandusky*, 2009; *Oikawa et al.*, 1995; *Tanaka et al.*, 1996] or biodegradation by-products such as 6-hydroxy-2-MIB, 5-hydroxy-2-MIB, 3-hydroxy-2-

MIB and 5-keto-2-MIB produced by camphor-degrading bacteria [*Eaton and Sandusky*, 2009].

The capability to degrade geosmin was proven for some members of α-Proteobacteria [*Ho et al.*, 2007; *Hoefel et al.*, 2006]. In Eagle Creek Reservoir, the strongest and more robust correlations were linked to the presence of two α-Proteobacteria: *Novosphingobium hassiacum* and *Sphingomonas oligophenolica*. Biodegradation by a consortium of gram negative bacteria [*Hoefel et al.*, 2006] can also be suggested in Eagle Creek Reservoir if the activity of *Sphingopyxis granuli* on cell-bound geosmin is considered for this cooperative work (Appendix II, r_s= 0.69; $p < 0.05$). The β-Proteobacteria *Comamonas odontotermitis* [*Guttman and van Rijn*, 2012] was the third identified player who may have contributed to the GSM biodegradation during phase I. Major by-products of the biodegradation of geosmin have been identified as 2-ketogeosmin and 7-ketogeosmin as well as several other minor products [*Eaton and Sandusky*, 2010]. Unfortunately, none of these by-product compounds were monitored throughout the 2013 sampling campaign. In order to substantiate the biodegradation of geosmin in natural environments, it is not excluded that the growth of some bacteria identified in the current study could have also been based on occurrences of these two ketogeosmins.

The importance of B:D ratios

The seasonal variations of MIB and GSM concentrations are the response of producers to different environmental factors such as nutrient availability [*Peter et al.*, 2009], light [*Zhang et al.*, 2009] or the presence of lysing agents [*Velzeboer et al.*, 1995]. Steps of the growth phase also influence the fractionation of dissolved (extracellular) and cell-bound (intracellular) concentrations [*Rosen et al.*, 1992]. As seen in Table 4.1 and Figure 4.2, over the 2013 campaign, MIB was mostly dissolved in water, with a mean B:D ratio of 0.10. The pattern for GSM is completely different as it was mainly found cell-bound (mean B:D =0.61). Differences in behavior between MIB and GSM likely impact their availability as a food source for degraders. Dissolved compounds that leaked out of the cells are more likely to be volatilized and generate unpleasant odorous. Moreover, this dissolved fraction is more bioavailable to degraders, and would be taken up and disappear more rapidly from the environment. In the case of Eagle Creek Reservoir, many more MIB-

degrading bacteria were found in terms of OTU number and abundance (*e.g. Flavobacterium ssp.*) in response to the large availability of dissolved MIB. As mainly bound to cell membranes, GSM was less susceptible to biodegradation and remained longer in water until cell walls become disrupted by lysing factors (viruses, algaecides, grazers, senescence). In addition, only a few known GSM-degrading bacteria were detected in the reservoir and, abundances of *Novosphingobium* and *Sphingomonas* were approximately 5 to 10 times lower than *Flavobacterium* (Figure 4.4). Scarcer availability of dissolved GSM seemed to have not allowed the support of larger populations of potential degraders.

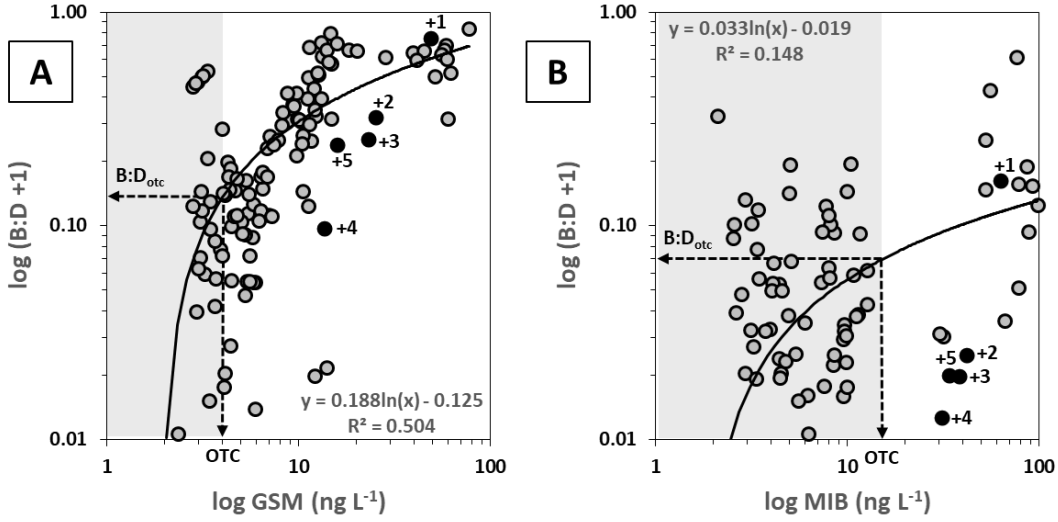

Figure 4.6: Relationship between Bound to Dissolved (B:D) ratios and A) Geosmin or B) MIB concentrations. The shaded area represents values below the Odor Threshold Concentration (OTC); with B:D$_{otc}$ as the intersect of OTC and the curve. Black and labelled dots highlight post- algaecide treatment days.

Interestingly, when geosmin concentrations increased, B:D ratios followed the same trend thus, meaning that most GSM was found intracellular or cell-bound (Figure 4.6A). The rise of geosmin in ECR was demonstrated to be linked to the growth of *Planktothrix* species during periods of reservoir mixing corresponding to phases I and III as described in this study and, to the growth of *Dolichospermum spp.* during the summer stratification or phase II [*cf.* Chapter 2]. From the equation displayed in Figure 4.6A, the calculation of the B:D ratio corresponding to the OTC of geosmin (B:D$_{otc}$) provided the value of 0.14 which indicates that beyond 14% of cell-bound GSM, increasing

concentrations of this compound tends to be more and more odorous. Unfortunately, the different behavior of MIB showed a poor correlation between dissolved and bound forms (Figure 4.6B). Low or high concentrations of MIB did not show a clear fractionation between dissolved and bound MIB. Lower than OTC concentrations revealed that MIB tended to be found more frequently in the dissolved fraction but the reverse was not true for higher levels with MIB being independently intra- or extracellular. After application of a copper-based algaecide on June 2nd 2013, both GSM and MIB concentrations dropped linearly (Figure 4.6) concurrently with their B:D ratios but went back up after the fifth day. This observed decrease was more drastic for GSM as *Planktothrix spp.* were highly impacted by the copper treatment while *Streptomyces spp.* (Actinobacteria) did not seem to be inflicted as much as Cyanobacteria were [*cf.* Chapter 3]. Nonetheless, the calculation of MIB B:D_{otc} from the entire dataset yielded a value of 0.07. The poor correlation tells why it is much lower than GSM and could be at least equivalent if higher concentrations of MIB were more frequently cell-bound. In the case of GSM, the B:D_{otc} of 0.14 can eventually be utilized as a reference value to determine whether the bacterial biodegradation will be triggered. Lower than B:D_{otc} values would indicate that dissolved GSM prevails and is more susceptible to degraders. From a manager's perspective, higher B:D_{otc} will be sought as good indicators to be implemented in the treatability index that will assist to prepare the water treatment process for removal optimization.

Conclusions

The 2013 sampling campaign of Eagle Creek reservoir witnessed two major outbreaks of T&O compounds. To describe the timeline of these outbreaks, three phases were identified based on intensities of the peaks and OTC exceedances of MIB and/or GSM. Geosmin outbreaks were always found with a prevalence of the cell-bound fraction or high B:D ratios during phases I and III. Conversely, this was not always the case for MIB which displayed high ratios for a short period of time during the first phase. Nevertheless, T&O outbreaks allowed to identify several potential degraders. Four Bacteroidetes identified as *Flavobacterium resistens, Flavobacterium granuli, Flavobacterium saliperosum* and *Flavobacterium kamogawaensis* showed strong

correlations with MIB occurrences and, two α-Proteobacteria *Novosphingobium hassiacum* and *Sphingomonas oligophenolica* with geosmin. The role of several *Pseudomonas spp.* was unclear as they showed affinities for both MIB and GSM. Even though individual contributions was impossible to determine, *Pseudomonas* species were very likely involved in the biodegradation. The fractionation of T&O compounds is an important parameter to consider as more dissolved compounds are crucial to assess bacterial biodegradation whereas high cell-bound T&O can be used by managers for optimal treatment adjustments.

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CHAPTER 5 – LOSS PROCESSES OF MIB AND GEOSMIN IN NATURAL LAKE SEDIMENTS

'Loss is nothing else but change, and change is Nature's delight'
Marcus Aurelius

Introduction

Sorption processes at the sediment-water interface play a crucial role on the transport, bio-availability and concentrations of nutrient [*Zhou et al.*, 2001], organic matter [*Huang et al.*, 2003; *Meyers and Ishiwatari*, 1993] and trace metals [*Traina and Laperche*, 1999; *Yuan et al.*, 2018] in lakes. Considered as a major repository for many organic and inorganic compounds, sorption processes onto natural sediments have been widely investigated [*Cornelissen et al.*, 2005; *Søndergaard et al.*, 2003; *Wang et al.*, 2007]. Sorption processes of inorganic P received the most attention as releases into water are critical for the growth of microorganisms such as algae and potentially toxic cyanobacteria [*Paerl et al.*, 2001]. Previous studies on sorption capacities in soils with different textures showed variations and tended to be higher when small size particles like clays were abundant [*De Willigen et al.*, 1982]. High proportions of iron/aluminum oxides enhance sorption capacities in clay-containing soils [*Herlihy and McGrath*, 2007; *Strahm and Harrison*, 2007] while clay minerals even have a greater binding capacity than Fe or Al oxides [*Gérard*, 2016]. In aquatic systems, sediment properties affect sorption processes [*Goldberg and Sposito*, 1985; *Walling and Moorehead*, 1987]. The size, the surface area and the weight of mineral particles are important characteristics which influence the sorption capacities of sediments [*Wang et al.*, 2006; *Warren and Zimmerman*, 1994]. The chemical composition of sediments can also affect the settling of microbial degraders, thus influencing their sorption capacities [*Sharpley et al.*, 1994]. Mineralization of organic matter (OM) is an important parameter to consider as it may change pH and redox potential [*Detenbeck and Brezonik*, 1991]. Humic acids are known to stabilize iron particles and promote the growth of Fe(III)-reducing bacteria [*Kappler et al.*, 2004; *Lovley et al.*, 1998]. Two major sources of dissolved organic carbon (DOC) have been identified in aquatic

ecosystems. The allochthonous origin consisting of terrestrial materials have already been extensively processed in soils. This DOC is deemed to be recalcitrant to further bacterial degradation [*Schiff et al.*, 1997]. The autochthonous source is the within-lake production by photosynthetic organisms like microalgae and macrophytes who release DOC into water through cell wall leakage, upon cell death by senescence, lysis by pathogens or by grazing predators [*Bertilsson and Jones*, 2003]. Autochthonous DOC is generally labile [*Søndergaard and Middelboe*, 1995] and is the preferred carbon source by bacteria [*Cole et al.*, 1988]. Many small organic compounds known as secondary metabolites are produced by aquatic bacteria in sediments [*Zuo et al.*, 2010] and, in water especially during blooms of cyanobacteria which can be toxic [*Kenefick et al.*, 1992; *Watson et al.*, 2008]. Toxic compounds in water, *i.e.* cyanotoxins, are well studied because of the potential human health risk of exposure [*Carmichael*, 2001; *Funari and Testai*, 2008]. Microcystin toxins are also found in pore water as well as in deeper sediments over 100 years old [*Zastepa et al.*, 2015]. However, fewer studies were conducted on the elimination by sorption process in natural sediments [*Rapala et al.*, 1994]. Microbial degradation of cyanotoxins is minor in sediments where losses by adsorption are prevalent [*Chen et al.*, 2008; *Rapala et al.*, 1994]. Sorption reactions of microcystins are pH dependent and sediment OM dependent: microcystins either compete for adsorption sites with organic matter in lower OM-containing sediments or interact with adsorbed OM in organic-rich sediments [*Wu et al.*, 2011]. The presence in sediments of dissolved OM promotes the degradation of cylindrospermopsin suggesting that some substrate specificity of OM may influence the degradation reaction [*Klitzke et al.*, 2010].

The fate and persistence of volatile Taste-and-Odor compounds, such as 2-methyl-isoborneol (MIB) and geosmin (GSM) are poorly understood in aquatic ecosystems despite the inconvenience these metabolites pose to the drinking water industry. As conventional water treatments are not efficient at removing T&O compounds [*Montiel*, 1983], for several decades the main core of studies have been focusing on granular or powdered activated carbons with enormous sorption capacity [*Cook et al.*, 2001; *Doederer et al.*, 2018; *Wnorowski*, 1992] in order to optimize removal rates during the water treatment process in plants [*Shang et al.*, 2018; *Zamyadi et al.*, 2015]. The effect of competition of MIB and geosmin with OM in natural waters was also assessed [*Graham et al.*, 2000].

Regarding the biodegradation process, water temperature is a critical factor that controls degrading bacteria. Optimal temperatures for effective MIB and GSM biodegradation range from 11 to 30ºC [*Christoffersen et al.*, 2002; *Elhadi et al.*, 2006; *Ho et al.*, 2007] and are correlated to the abundance of T&O-degrading bacteria [*Ho et al.*, 2007; *Hoefel et al.*, 2009]. Besides, elevated T&O concentrations in source waters tend to be met with higher rates of biodegradation [*Hoefel et al.*, 2009]. It has been suggested that water treatment plants' sand filters can be used to harbor T&O-degrading bacteria in order to enhance the bio-filtration process [*Ho et al.*, 2007; *Ho et al.*, 2012; *Hsieh et al.*, 2010].

The purpose of this study is to investigate the fate of MIB and geosmin in source waters. Retained concentrations of MIB and geosmin in sediment interstitial water are at the interface of sorption reactions and bacterial biodegradation. These two loss processes will be evaluated. The adsorption capacities of Eagle Creek Reservoir's sediments will be tested and compared to other adsorbents (bentonite and ferrihydrite) in order to determine their removal efficiencies for MIB and geosmin compounds

Materials and Methods

Study site

Eagle Creek Reservoir located in central Indiana (Figure 5.1), receives drainage from 419.6 km^2 of the Eagle Creek Watershed and has a surface area of 5.7 km^2. The reservoir was constructed in 1967 to provide flood control and then drinking water for the city of Indianapolis and surrounding communities. The maximum depth ranges from about 11 to 13 meters, with the deepest areas located in the southern basin, near the dam. Eagle Creek Reservoir is a small, dimictic, and eutrophic water body with seasonal thermal stratification from June to September. Reservoir mixings usually occur in April/May and October each year. The mean annual discharge of Eagle Creek, the main tributary, is 35.74 $m^3.s^{-1}$ with maxima recorded between April and June. The calculated residence time of the reservoir is 39.5 days.

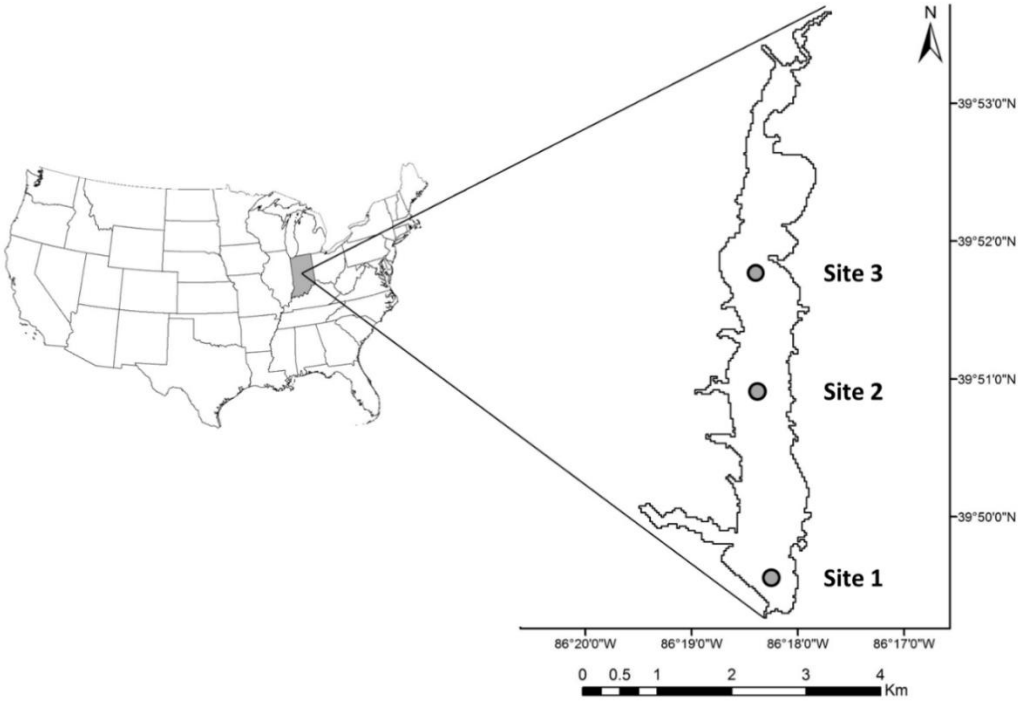

Figure 5.1: Locations of Eagle Creek Reservoir's sediment cores sampling sites.

Sediment collection and processing

Sediment collection – Sediment cores were collected at three different locations in the reservoir. Site 1 represents the deepest area near the dam with an average depth of 10.6 meters. Site 2 is the mid-reservoir with 6.5 m of depth and, site 3 represents the upper basin of Eagle Creek Reservoir (Figure 5.1). The shallow upper basin receives direct drainage from the main tributary Eagle Creek. The average depth at this sampling location is 3.2 meters. A total of six cores were collected: cores I-IV in May, core V in June and core VI in August 2015 (Table 5.1). A gravity corer was used to collect sediments and then brought back to the lab to be extruded. Each time, the top layer corresponding to the sediment-water interface was scraped off with a cutting plate and kept frozen until further analysis.

Grain size analysis – The top centimeter of sediment cores were sectioned to analyze the grain size of settled particles at the sediment-water interface. Wet samples were poured over a 125 µm sieve and rinsed with DI water for gross organic matter removal. Dump content was brushed with a small paintbrush to get rid of the smaller visible organic detritus. Then, samples were boiled and oxidized with 30% of H_2O_2 for 15 minutes to

remove remaining organic matter. The cycle was repeated twice to minimize the amount of organic matter left. Clean sediments were re-suspended in 500 mL DI water and allowed to settle overnight. Top water was siphoned off and collected materials (n=5; 52.18 ± 6.02 g) were put into 50-mL plastic Falcon™ tubes and centrifuged at 3,500 rpm for 5 minutes and then, 8,000 rpm for 10 minutes. Top water was poured off and 20 mL of sodium hexa-metaphosphate was added to each sample tube as a dispersing agent. Samples were then shaken for 30 seconds on a mechanical shaker. Naturally cemented or aggregated particles were separated with the assistance of the sodium meta-phosphate which prevented the flocculation of the dispersed particles. Finally, suspended sediment particles were ready for subsequent determination on a Malvern Mastersizer 2000 particle-size analyzer (Malvern Instruments, UK). Average percentages of clays, silts and sands were obtained from three replicates per sediment core.

Sediment desiccation and loss on ignition – To characterize the moisture content of each sample, wet sediment splits were weighted prior to desiccation at 60ºC for 24 hours and then, dry weights were measured. The difference of masses was calculated to estimate the mass of interstitial water. The content of organic matter was measured by the loss on ignition (LOI) method. Desiccated sediments were placed in pre-weighted porcelain crucibles and then combusted in a muffle furnace at 550ºC for 2 hours. The measured value obtained from LOI was divided by 2.44 to determine the organic matter content [*Jacinthe et al.*, 2010].

Sorption experiments

Interstitial MIB and geosmin – Known masses of sediments (n= 27; 508.7 ±10.9 mg) were put into 120-mL glass jars and filled with 50 mL of DI water (18MΩ). Samples were left at room temperature on lab countertops without any shaking. Triplicate samples were collected through time, between 5 minutes and 5 days, for measurements of MIB and geosmin in water. Sample blanks with no sediments in jars were run at 60 minutes after the beginning of the experiment.

Adsorption experiment – 50 mL of a spike solution of MIB (94.1 ng L^{-1}) and geosmin (95.0 ng L^{-1}) were added to desiccated sediments (n= 27; 524.5 ±19.5 mg) in glass amber vials with a rubber septum. Samples were shaken continuously on a Labquake™

tube rotator at 60 rpm until the end of the experiment. Triplicate samples were collected for MIB and geosmin analysis at given time intervals between 90 minutes and 28 days.

Sorption comparison – The sorption capacity of MIB and geosmin was tested on two other minerals, such as bentonite which is an aluminum phyllosilicate clay and ferrihydrite produced from oxidation of Mohr's salt as ammonium iron (II) sulfate hexahydrate (Sigma Aldrich). The same stock solutions as the previous experiment (see above adsorption experiment) were used. Meanwhile, sodium azide (0.1% N_3Na; Fisher Scientific) was added as a biocide to inhibit the bacterial activity that could occur in sediment samples. Sorption capacities were also compared between the three Eagle Creek Reservoir sampling sites. A glassware blank with no sediment was also run. All samples were measured for MIB and geosmin after the 10^{th} day of experiment.

T&O analysis

Methylisoborneol and geosmin concentrations were quantified by a Head-Space Solid-Phase Micro-Extraction (HS-SPME) combined with a Gas Chromatography-Mass Spectrometry (GC-MS) to analyze the volatile and odorous compounds [*APHA*, 2000].

Statistical test

To compare removal capacities of MIB and geosmin between samples, a one-way ANOVA and a Tukey *post hoc* test were run, using Past 3.1 software [*Hammer et al.*, 2001], to highlight significant differences in sorption capacities between adsorbent materials.

Results

Eagle Creek Reservoir granulometry

A total of six sediment cores were collected in the reservoir. Site 1, near the dam, had the largest fraction of clays compared to the other cores. Site 2, from the middle reservoir, had the most sands (17.3%) as the corer hit the old *talweg* of the main tributary (Table 5.1). The four sediment cores collected in the upper basin of the reservoir (Site 3) were relatively homogenous in terms of grain size composition (Figure 5.2). On average,

silts represent the largest fraction with 70.2%, clays 26.7% and sands 3.1% (Table 5.1). The core texture is described as '*silt loam'* typical of highly fertile agricultural soils which retain water, *i.e.* alfisols, commonly encountered in the US Midwest [*Staff*, 1999]. After desiccation, bulk sediment cores contained in average 53.03% (Site 3), 48.65% (Site 2) and 35.11% (Site 1) of water. This longitudinal gradient in pore space can be explained by the increasing amount of impervious clays from the upper basin to the dam. The loss on ignition analysis revealed a reverse gradient regarding the content of organic matter in sediments. The upper basin cores showed the highest content with 0.31%, mid-reservoir with 0.27% and dam with 0.20% of OM (Table 5.1).

Table 5.1: Bulk mineral composition of sediment cores, moisture and organic matter (OM) content are expressed as weight percent units.

	Site 1	**Site 2**	**Site 3**				
	Core I	Core II	Core III	Core IV	Core V	Core VI	Average
% Clays	**46.0**	**33.8**	**27.3**	**25.6**	**27.8**	**26.1**	**26.7**
% Silts	**52.6**	**48.9**	**71.0**	**70.9**	**69.0**	**70.1**	**70.2**
% Sands	**1.4**	**17.3**	**1.7**	**3.5**	**3.2**	**3.8**	**3.1**
Total	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**
wt% Moisture	**35.11**	**48.65**	**52.05**	**54.02**	**-**	**-**	**53.03**
wt% OM	**0.20**	**0.27**	**0.32**	**0.30**	**-**	**-**	**0.31**

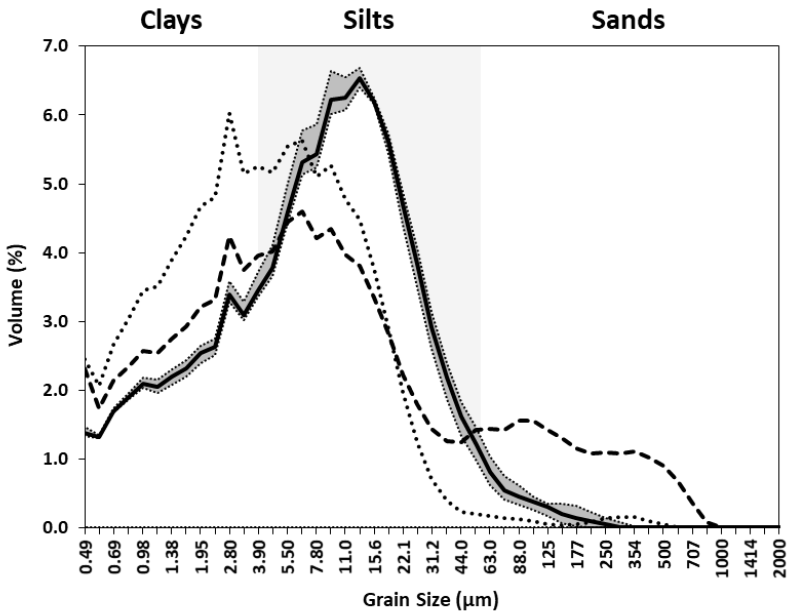

Figure 5.2: Grain size analysis of Eagle Creek Reservoir sediments; Site 1 (bold dotted line), Site 2 (dashed line) and Site 3 with the median value (bold black line), 10th percentile (lower dotted line) and 90th percentile (upper dotted line). Shaded area represents the silt fraction.

Interstitial and desorbable T&O compounds

The first experiment was conducted in order to verify if there was any MIB or geosmin within the pore water of Eagle Creek sediments. T&O compounds that leaked out of interstices were rapidly detected in the DI water medium after 5 minutes; with an average concentration of 15.61± 1.73 and 5.90± 1.38 ng L^{-1}, for MIB and geosmin respectively (Figure 5.3). This desorption process led to a combination of interstitial and desorbable MIB and GSM. Detected concentrations remained constant for about a day and then began to decrease below detection levels on day 3.

Sorption of MIB and Geosmin

The dynamics of initial concentrations of MIB and geosmin from the stock solution to the sediment were studied through time. The primary goal was to assess whether T&O compounds can sorb onto sediments. On Figure 5.4, it is clear that geosmin quickly disappears from the solution after 90 minutes of contact time whereas there is a lag phase for MIB whose removal is triggered between 0.5 and 0.75 day (12 – 18 hours). Calculations

derived from equations indicate that total geosmin is completely removed from the solution after 15.4 days. The sorption process of MIB is slower and compounds are entirely removed after 37.8 days.

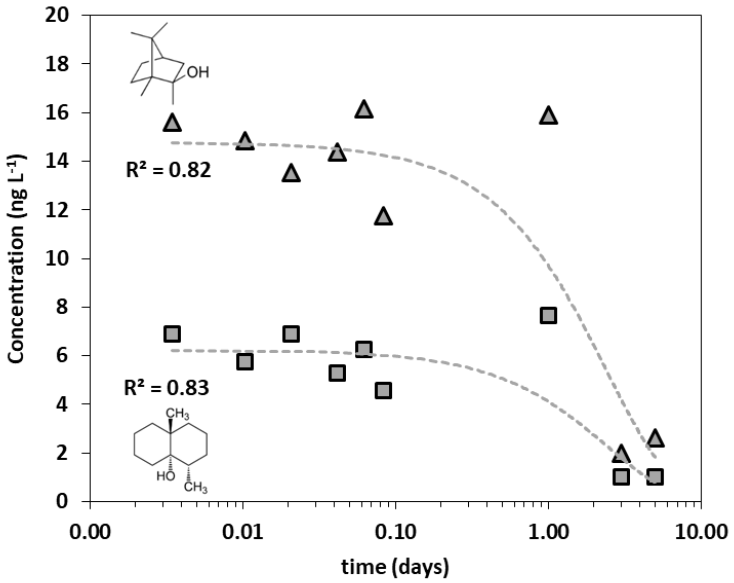

Figure 5.3: Desorption of interstitial MIB (triangles) and geosmin (squares) in DI water from natural sediments of Eagle Creek Reservoir.

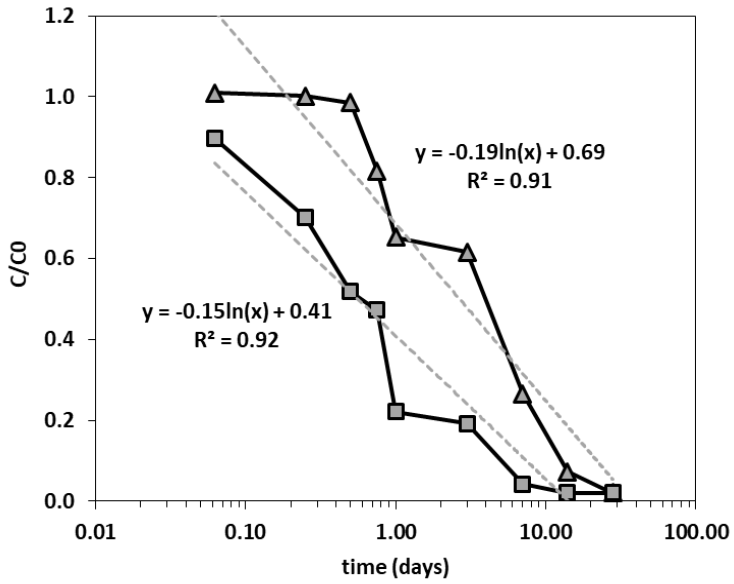

Figure 5.4: Sorption of MIB (triangles; C_0= 94.1 ng L^{-1}) and geosmin (squares; C_0= 95.0 ng L^{-1}) onto Eagle Creek Reservoir sediments.

Comparison of removal capacities

There was a statistically significant difference between MIB and geosmin removal efficiencies by natural sediment and other adsorbents as determined by one-way ANOVA (F= 29.46; $p < 0.001$). The Tukey *post hoc* test revealed that there was no statistically significant differences between the glassware blank and azide control ($p = 0.974$), azide + sediment ($p = 0.971$) and, between azide control and azide + sediment ($p = 1$). This indicates that MIB and geosmin concentrations remained the same in jars after 10 days of contact time with the biocide. A significant difference was also observed between bentonite and ferrihydrite ($p < 0.05$), site 1 ($p < 0.05$) whereas sites 2 and 3 were non-significant. The sorption efficiency of ferrihydrite was similar as sites 1, 2 and 3 ($p = 1$). Bentonite's removal capacity of MIB and geosmin is lower than ferrihydrite and Eagle Creek Reservoir natural sediments, especially site 1 which contains the highest amount of clays (Table 5.2). Bentonite and ferrihydrite are better at removing MIB than GSM contrary to ECR sediments which are more efficient at GSM removal.

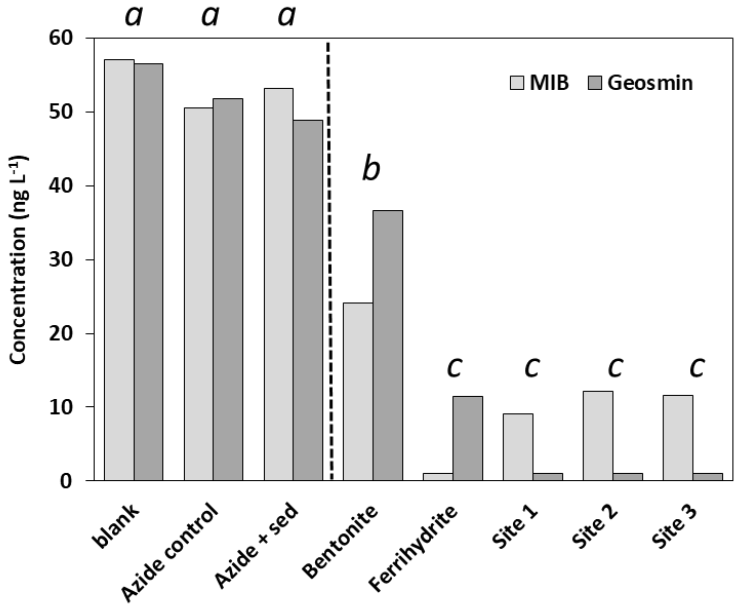

Figure 5.5: Comparison of MIB and geosmin removal by different adsorbents: bentonite (clays), ferrihydrite (oxy-hydroxides) and *'silty loamy'* sediments from Eagle Creek Reservoir (see Figure 5.2 for details). Blank and Azide control have no sediments; sodium azide treatment (0.1%). Statistically significant differences are expressed by different letters.

Table 5.2: Comparison of removal efficiencies of MIB and geosmin by different absorbent materials; with Ci: initial concentration and Cf: final concentration.

	Blank	Azide	Azide + Sed	Bentonite	Ferrihydrite	Site 1	Site 2	Site 3
mass (g)	0.000	0.000	0.548	0.548	0.143	0.554	0.542	0.527
MIB								
Ci	94.1	94.1	94.1	94.1	94.1	94.1	94.1	94.1
Cf	57.1	50.6	53.2	24.1	1.0	9.1	12.2	11.7
% removal	39.3	46.3	43.5	74.4	98.9	90.3	87.0	87.6
Geosmin								
Ci	95.0	95.0	95.0	95.0	95.0	95.0	95.0	95.0
Cf	56.6	51.8	48.9	36.7	11.4	1.0	1.0	1.0
% removal	40.5	45.5	48.5	61.4	88.0	98.9	98.9	98.9

Table 5.3: Estimated quotas of T&O losses from Eagle Creek Reservoir sediments. Volatilization was calculated as the difference between final concentrations of MIB and GSM in jars minus losses by sorption, bacterial degradation and the dissolved fraction.

Percent (%)	MIB	GSM
Volatilization	39.3	40.5
Sorption	4.1	8.0
Bacterial	44.8	50.4
Dissolved	11.8	1.1

Discussion

The experiments conducted in this study on Eagle Creek Reservoir sediments provide important information regarding the fate of MIB and geosmin compounds in water. First, average concentrations of 15.61 ng L^{-1} of MIB and 5.90 ng L^{-1} of geosmin were extracted from interstitial water. These concentrations remained stable for about one day and then rapidly decreased to non-detect levels. This abrupt changes may denote the activation of the bacterial breakdown of T&O compounds as collected sediment samples were not sterile and were seeded with their own natural microbial flora (Figure 5.3). Next, the sorption experiment of T&O compounds onto the reservoir's sediments showed that geosmin began to be removed from the medium after 90 minutes of contact time whereas MIB removal was triggered after 12-18 hours. The sharp decrease after day 1 may match the same drop observed in the interstitial water experiment, *i.e.* the triggering of microbial

degraders' growth. Nevertheless, the complete removal process by natural sediments is slow; removal of geosmin was achieved after 15 days and MIB after 37 days (Figure 5.4). These results do not allow us to discriminate between chemical sorption and bacterial breakdown of MIB and geosmin. However, it is clear that geosmin disappears more rapidly from the medium whereas, in general, a lag phase of about one day is required for MIB. Finally, after 10 days of incubation, the blank sample displayed *ca.* 40% removal of both compounds compared to initial concentrations of stock solution (Table 5.2). This loss was due to volatilization of MIB and geosmin. Sediment samples treated with sodium azide showed a +4.1% (MIB) and +8.0% (geosmin) loss compared to blank samples (Figure 5.5). This observed loss of MIB and GSM points out at the chemical sorption of T&O compounds onto sediments. The calculated difference of T&O final concentrations between ECR sites' samples (1, 2 and 3) and azide + sediment samples leads to the loss by 'bacterial degradation' which is estimated around 45% (MIB) and 50% (Geosmin). The remaining quota corresponds to the amount of T&O compounds left dissolved in the medium, *i.e.* <12 ng L^{-1} for MIB and <2 ng L^{-1} (below detection limit; BDL) for geosmin (Table 5.3). Amongst adsorbent materials, bentonite had the lowest removal efficiency but both bentonite and ferrihydrite were more efficient at removing MIB. Reversely, all natural sediments from Eagle Creek Reservoir described as a mixing of clays and silts were better at removing geosmin (BDL) while *ca.* 10 ng L^{-1} of MIB remained in the medium (Figure 5.5). These loss mechanisms explain the previous observations of T&O levels in the reservoir's raw water; *i.e.* quick disappearance of geosmin and remaining dissolved MIB [*cf.* Chapters 2 & 4].

Conclusions

Natural sediments retain T&O compounds in pore waters. These compounds can sorb onto mineral particles but this loss process is very slow and limited. Bacterial breakdown of MIB and geosmin is more likely the prevalent mechanism which contributes to the largest loss of T&O compounds. The silty loamy composition of Eagle Creek sediments tends to favor microbial degradation over chemical sorption which seems to be

more effective at geosmin removal than MIB. In opposition, Al/Fe oxides from bentonite and ferrihydrite are more effective to remove MIB.

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CHAPTER 6 – CONCLUSIONS & PERSPECTIVES

Occurrences of T&O-producing organisms are common in surface waters. Seasonal blooms of bacteria can rapidly plague a drinking water supply and subsequently, the biosynthesis of elevated concentrations of odorous metabolites such as MIB and Geosmin can easily impair drinking water quality. In the first chapter, the study identified the environmental factors that were important triggers for the growth of MIB- and GSM-producing bacteria in Eagle Creek Reservoir. Cool water temperatures during spring time concurrently with high stream discharges from Eagle Creek bringing in high levels of nitrogen and mixing the whole reservoir were identified to generate favorable conditions to support the growth of *Streptomyces* (Actinobacteria) and *Planktothrix* (Cyanobacteria) involved in the *in situ* production of MIB and geosmin, respectively. High T&O levels in the reservoir waters were estimated to respond to Eagle Creek peak discharges that occurred 37 days earlier. Hence, it has been inferred that peaks in production of both odorous metabolites were detected when the duration of this lag period was shorter than the reservoir residence time. In the second chapter, the use of a shotgun sequencing technique documented the bacterial assemblages of Eagle Creek Reservoir during severe T&O outbreaks. Based on 16S rRNA gene detections, the bacterioplankton community was dominated in order of higher abundances by Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes and Cyanobacteria. Main producers of MIB were identified as *Streptomyces* and *Micromonospora* (Actinobacteria) and, for geosmin as *Anabaena* and *Planktothrix* (Cyanobacteria). Surprisingly, in Eagle Creek Reservoir, a clear and sharp separation in metabolite biosynthesis was demonstrated; MIB seemed to be exclusively produced by Actinobacteria and GSM by Cyanobacteria although *Streptomyces* might potentially be involved in the production of both metabolites. The spatial analysis of bacterioplankton distribution showed that T&O-producing species were located in the upper layers of the water column (0-3m) which coincided with the highest detections of precursor enzymes involved in the metabolic pathways of mono- and sesquiterpenes. Applications of a copper-based algaecide to curtail the T&O production in the reservoir impacted bacteria differently. Cyanobacteria tended to be more sensitive than Actinobacteria and, as a consequence the production of geosmin was immediately

terminated whereas moderate MIB detections lingered. This difference in behavior between odorous compounds was detailed in the third chapter. After cellular release, fractionation analyses of the metabolites showed that MIB was more frequently detected in the dissolved fraction while geosmin was essentially cell-bound. This discovery was determinant as it now sheds light on the susceptibility of each metabolite to bacterial degradation. Investigations conducted in the epilimnion during T&O outbreaks led to bacteria identified as potential degraders; with high dissolved MIB linked to higher abundances of four *Flavobacterium* species (Bacteroidetes) and cell-bound geosmin linked to *Novosphingobium* and *Sphingomonas* species (α-Proteobacteria). *Pseudomonas* species may have played an ambiguous role and could have been involved in the biodegradation of both compounds. The last chapter of the present study was intended to characterize the loss processes of T&O compounds. Sorption experiments showed that MIB and GSM can sorb onto Eagle Creek Reservoir's natural sediments but that loss mechanism was minor and very slow. Main losses of T&O metabolites were mainly due to bacterial breakdown within the sediments which contributed to the removal of at least half of the initial T&O concentrations while the chemical adsorption represented less than 8%.

The original design of the study presented in this research work was to identify and understand the major mechanisms involved in the production of MIB and geosmin in a water supply reservoir. Although hydrological drivers and water temperatures were found critical, the accurate identification of organisms involved in the *in situ* production of odorous metabolites is still evolving and challenging. This step was achieved with the help of Illumina next-generation sequencers (NGS) and extensive reference databases for taxonomic assignments. Shotgun genomics based on 16S rRNA analysis revealed the presence of all bacterial taxa in the same water sample (*i.e.* environmental DNA or eDNA); thus, simultaneously screening for taxonomic and functional diversities. This DNA-based approach is sometimes referred to as "DNA metabarcoding". However, this expression is more appropriate when pointing at taxa identification while 'metagenomics' refers to functional aspects and genome assemblies. Now that major producers have been identified in Eagle Creek Reservoir, the continuation of this work will be to determine accurately the presence or absence of a single species, such as *Streptomyces* or *Planktothrix*. A species-

specific approach is very often the best solution and is generally based on quantitative Polymerase Chain Reaction (qPCR) or targeted PCR. These two techniques target a metabarcode, *i.e.* a short and informative DNA region flanked by two conserved regions which harbor PCR primers. Such primers already exist to detect major bacterial clades but must be first clearly defined to aim specifically at the group of interest. Furthermore, existing metabarcodes may not be entirely satisfying and *in silico* primer design is often an alternative approach to refine the taxonomic resolution. Similarly, the designing of primers that flank genes encoding for MIB or geosmin is utterly relevant and possible. The sole prerequisite for *in silico* designing of a robust and efficient metabarcode is to get access to a set of reference sequences that truly represent the group/gene of study with the lowest levels of sequencing errors. Once a good set of sequences is obtained, primer anchoring regions may emerge and be identified from sequence alignments or, instead, based on a pattern search. The high specificity of these newly designed primers is to ensure that DNA from non-target organisms will not be co-amplified. Then, MIB and GSM-producing bacteria can be monitored routinely in Eagle Creek Reservoir. A real-time biomonitoring program may as well be set up to forecast and anticipate T&O outbreaks and potential water treatment impairments when environmental conditions are becoming favorable for the biosynthesis of odorous metabolites.

The advancements in knowledge regarding the behavior and the fate of MIB and geosmin in surface waters, from source to sink, presented in this research work are important from a water manager's perspective. Hydrological drivers and nitrate levels are the major component as they dictate whether conditions are favorable to support the growths of bacterial producers. These parameters can now easily be monitored online through the USGS super gages located on main tributaries upstream the reservoir at Zionsville, IN (USGS 03353200) and recently, at Brownsburg, IN (USGS 03353420). The list of known T&O-producing bacteria is growing every day. Major metabolic pathways are being unraveled and biological controls may be proposed in a near future. The comprehension of cell-bound to dissolved fractions may be a useful parameter to decide for optimal treatment/cost adjustments within the water treatment plant as well as whether to decide or not to treat the reservoir with an algaecide depending on which metabolite is

present in the water at a given time. Finally, it is not impossible that the identification of new bacterial species or strains capable of degrading T&O compounds may have future applications in the water industry and, as well be used to seed sand filter beds in order to enhance the removal efficiency of treatment plants.

SUPPLEMENTAL TABLES S1-S6

Table S1. Formulas used to calculate diversity indices; with *pi* the proportion of individuals belonging to species *i*.

Metric	Traditional formula
Richness (S)	Number of species
Shannon's diversity (H')	$-\sum pi \ln(pi)$
Simpson's dominance (D)	$1/\sum pi^2$
Simpson's evenness (E)	D/S

Table S2. Variations of diversity indices of bacterioplankton with depths in the Eagle Creek water column and seasons.

	Spring				Summer				Fall		
	0m	**3m**	**6m**	**10m**	**0m**	**3m**	**6m**	**10m**	**3m**	**6m**	**10m**
Richness S	590	592	593	592	592	589	590	592	590	594	590
Shannon H'	3.79	4.21	3.36	3.08	3.98	3.14	2.83	4.74	2.89	2.75	2.74
Dominance D	0.07	0.08	0.16	0.23	0.06	0.13	0.23	0.04	0.25	0.25	0.27
Evenness E	0.075	0.114	0.049	0.037	0.090	0.039	0.029	0.193	0.031	0.026	0.026

Table S3. Top 5 most abundant bacterial phyla in each Eagle Creek Reservoir water sample.

	Spring				Summer				Fall		
	0m	**3m**	**6m**	**10m**	**0m**	**3m**	**6m**	**10m**	**3m**	**6m**	**10m**
# 16S reads	7,748,921	8,578,024	13,881,256	9,161,193	5,802,341	19,669,842	17,593,523	4,531,446	10,097,700	15,079,509	12,969,708
% Actinobacteria	75.3	60.4	83.6	83.9	36.2	39.2	56.4	24.8	70.6	70.7	74.5
% Proteobacteria	9.7	28.3	10.9	10.6	26.0	39.3	30.1	40.9	15.9	19.5	15.2
% Firmicutes	10.9	5.5	2.0	1.6	19.8	8.6	5.7	14.8	6.9	4.0	4.4
% Bacteroidetes	1.6	2.2	1.2	1.2	4.8	5.9	3.5	7.3	0.8	0.6	0.7
% Cyanobacteria	1.0	0.8	0.5	1.0	7.5	2.3	1.2	2.6	2.8	1.8	2.4
% Other bacteria	1.5	2.8	1.7	1.6	5.6	4.7	3.1	9.7	3.1	3.4	2.8

Table S4. Seasonal averages of enzyme reads from metabolic pathways and environmental variables, including odorous compounds MIB and geosmin, recorded in Eagle Creek Reservoir.

	Spring 2013				Summer 2013				Fall 2013			
Enzymes	**n**	**Mean**	**Max**	**St. Dev**	**n**	**Mean**	**Max**	**St. Dev**	**n**	**Mean**	**Max**	**St. Dev**
AACT [EC 2.3.1.9]	4	2526.8	3570.0	730.3	4	2799.0	4547.0	1897.8	3	3523.3	4258.0	777.6
HMGS [EC 2.3.3.10]	4	8.5	18.0	6.4	4	22.5	47.0	19.8	3	11.3	24.0	11.0
HMGR [EC 1.1.1.34]	4	71.5	82.0	13.2	4	72.8	102.0	22.9	3	40.0	46.0	8.7
MVK [EC 2.7.1.36]	4	11.3	22.0	8.1	4	9.0	15.0	5.5	3	1.0	3.0	1.7
PMK [EC 2.7.4.2]	4	6.3	17.0	7.5	4	3.5	12.0	5.7	3	0.0	0.0	0.0
MVD [EC 4.1.1.33]	4	9.5	14.0	3.7	4	3.3	6.0	2.8	3	0.3	1.0	0.6
dxs [EC 2.2.1.7]	4	2598.0	3910.0	1073.4	4	2803.5	4538.0	1725.3	3	3506.0	3974.0	541.4
dxr [EC 1.1.1.267]	4	697.0	1019.0	241.1	4	666.3	1060.0	332.4	3	581.0	628.0	58.8
mct [EC 2.7.7.60]	4	4.8	7.0	3.3	4	0.8	2.0	1.0	3	1.7	5.0	2.9
cmk [EC 2.7.1.148]	4	592.5	929.0	285.1	4	561.3	923.0	352.7	3	643.3	762.0	184.3
hdr [EC 1.17.1.2]	4	463.5	742.0	223.3	4	565.5	722.0	126.7	3	278.7	296.0	22.7
IDI [EC 5.3.3.2]	4	192.3	229.0	32.7	4	241.5	442.0	146.5	3	110.7	140.0	29.5
DMAPP [EC:2.5.1.1]	4	242.0	325.0	77.1	4	257.8	286.0	38.4	3	134.3	159.0	22.5
FPS [EC 2.5.1.10]	4	31.5	47.0	15.6	4	25.3	37.0	8.7	3	21.0	28.0	6.6
Environment												
MIB (ppt)	4	64.3	120.9	63.6	4	5.3	15.3	6.7	3	12.6	13.8	1.1
Geosmin (ppt)	4	27.8	51.4	26.3	4	3.4	3.9	0.4	3	15.1	20.8	5.0
NO_3-N (ppm)	4	1.0	1.3	0.2	4	1.0	1.5	0.6	3	0.0	0.0	0.0
NH_3-N (ppm)	4	0.2	0.7	0.3	4	0.7	2.4	1.1	3	2.0	5.4	3.0
Total P (ppm)	4	0.1	0.1	0.0	4	0.2	0.7	0.3	3	0.3	0.7	0.4
Temp (ºC)	4	16.7	14.0	5.2	4	22.4	34.8	5.9	3	19.4	55.0	2.1
pH	4	8.3	21.1	0.6	4	7.9	26.5	0.6	3	7.3	20.7	0.4

Table S5. List of OTUs identified in networks.

Phylum	Genus	OTU	Phylum	Genus	OTU
Actinobacteria	*Acidimicrobium*	*Acti-1*	**Actinobacteria**	*Propionibacterium*	*Acti-45*
Actinobacteria	*Acidothermus*	*Acti-2*	**Actinobacteria**	*Renibacterium*	*Acti-46*
Actinobacteria	*Actinomyces*	*Acti-3*	**Actinobacteria**	*Rhodococcus*	*Acti-47*
Actinobacteria	*Actinosynnema*	*Acti-4*	**Actinobacteria**	*Rothia*	*Acti-48*
Actinobacteria	*Aeromicrobium*	*Acti-5*	**Actinobacteria**	*Rubrobacter*	*Acti-49*
Actinobacteria	*Amycolatopsis*	*Acti-6*	**Actinobacteria**	*Saccharomonospora*	*Acti-50*
Actinobacteria	*Arcanobacterium*	*Acti-7*	**Actinobacteria**	*Saccharopolyspora*	*Acti-51*
Actinobacteria	*Arthrobacter*	*Acti-8*	**Actinobacteria**	*Salinispora*	*Acti-52*
Actinobacteria	*Atopobium*	*Acti-9*	**Actinobacteria**	*Sanguibacter*	*Acti-53*
Actinobacteria	*Beutenbergia*	*Acti-10*	**Actinobacteria**	*Scardovia*	*Acti-54*
Actinobacteria	*Bifidobacterium*	*Acti-11*	**Actinobacteria**	*Segniliparus*	*Acti-55*
Actinobacteria	*Brachybacterium*	*Acti-12*	**Actinobacteria**	*Slackia*	*Acti-56*
Actinobacteria	*Brevibacterium*	*Acti-13*	**Actinobacteria**	*Stackebrandtia*	*Acti-57*
Actinobacteria	*Catenulispora*	*Acti-14*	**Actinobacteria**	*Streptomyces*	*Acti-58*
Actinobacteria	*Cellulomonas*	*Acti-15*	**Actinobacteria**	*Streptosporangium*	*Acti-59*
Actinobacteria	*Clavibacter*	*Acti-16*	**Actinobacteria**	*Thermobifida*	*Acti-60*
Actinobacteria	*Collinsella*	*Acti-17*	**Actinobacteria**	*Thermobispora*	*Acti-61*
Actinobacteria	*Conexibacter*	*Acti-18*	**Actinobacteria**	*Thermomonospora*	*Acti-62*
Actinobacteria	*Corynebacterium*	*Acti-19*	**Actinobacteria**	*Tropheryma*	*Acti-63*
Actinobacteria	*Cryptobacterium*	*Acti-20*	**Actinobacteria**	*Tsukamurella*	*Acti-64*
Actinobacteria	*Dermacoccus*	*Acti-21*	**Actinobacteria**	Unclassified Actino-	*Acti-65*
Actinobacteria	*Eggerthella*	*Acti-22*	**Actinobacteria**	*Xylanimonas*	*Acti-66*
Actinobacteria	*Frankia*	*Acti-23*	**Cyanobacteria**	*Acaryochloris*	*Cyan-1*
Actinobacteria	*Gardnerella*	*Acti-24*	**Cyanobacteria**	*Anabaena*	*Cyan-2*
Actinobacteria	*Geodermatophilus*	*Acti-25*	**Cyanobacteria**	*Arthrospira*	*Cyan-3*
Actinobacteria	*Gordonia*	*Acti-26*	**Cyanobacteria**	*Crocosphaera*	*Cyan-4*
Actinobacteria	*Intrasporangium*	*Acti-27*	**Cyanobacteria**	*Cyanobium*	*Cyan-5*
Actinobacteria	*Janibacter*	*Acti-28*	**Cyanobacteria**	*Cyanothece*	*Cyan-6*
Actinobacteria	*Jonesia*	*Acti-29*	**Cyanobacteria**	*Cylindrospermopsis*	*Cyan-7*
Actinobacteria	*Kineococcus*	*Acti-30*	**Cyanobacteria**	*Cylindrospermum*	*Cyan-8*
Actinobacteria	*Kocuria*	*Acti-31*	**Cyanobacteria**	*Gloeobacter*	*Cyan-9*
Actinobacteria	*Kribbella*	*Acti-32*	**Cyanobacteria**	*Lyngbya*	*Cyan-10*
Actinobacteria	*Kytococcus*	*Acti-33*	**Cyanobacteria**	*Microcoleus*	*Cyan-11*
Actinobacteria	*Leifsonia*	*Acti-34*	**Cyanobacteria**	*Microcystis*	*Cyan-12*
Actinobacteria	*Micrococcus*	*Acti-35*	**Cyanobacteria**	*Nodularia*	*Cyan-13*
Actinobacteria	*Micromonospora*	*Acti-36*	**Cyanobacteria**	*Nostoc*	*Cyan-14*
Actinobacteria	*Mobiluncus*	*Acti-37*	**Cyanobacteria**	*Oscillatoria*	*Cyan-15*
Actinobacteria	*Mycobacterium*	*Acti-38*	**Cyanobacteria**	*Prochlorococcus*	*Cyan-16*
Actinobacteria	*Nakamurella*	*Acti-39*	**Cyanobacteria**	*Raphidiopsis*	*Cyan-17*
Actinobacteria	*Nocardia*	*Acti-40*	**Cyanobacteria**	*Synechococcus*	*Cyan-18*

Actinobacteria	*Nocardioides*	*Acti-41*	**Cyanobacteria**	*Synechocystis*	*Cyan-19*
Actinobacteria	*Nocardiopsis*	*Acti-42*	**Cyanobacteria**	*Thermosynechococcus*	*Cyan-20*
Actinobacteria	*Olsenella*	*Acti-43*	**Cyanobacteria**	*Trichodesmium*	*Cyan-21*
Actinobacteria	*Parascardovia*	*Acti-44*	**Cyanobacteria**	Unclassified Cyano-	*Cyan-22*

Table S6. Comparison of nucleotide sequences encoding for enzymes from the MEP/DOXP pathway belonging to the marine *Trichodesmium erythraeum* (Refseq Accession: YP_723652.1) and percent identity using the online NCBI BlastN Suite.

Sequence length (nt)	***Trichodesmium erythraeum* Contig ID**	**Function**	**Enzyme**	**E-value**	**NCBI taxon (% Identity)**	**Accession**
150	NS500123:11:H0E9PAGXX:1:12301:5238:15375.1	1-deoxy-D-xylulose-5-phosphate synthase	2.2.1.7	$2e^{-44}$	*Pseudanabaena sp.* (89%)	AP017560.1
118	NS500123:11:H0E9PAGXX:4:22410:20424:11333	2-C-methyl-D-erythritol 4-phosphate cytidylyl-transferase	2.7.7.60	$2e^{-53}$	*Planktothrix agardhii* (100%)	LO018304.1
252	NS500123:11:H0E9PAGXX:2:11210:21598:15131	4-diphospho-cytidyl-2-C-methyl-D-erythritol kinase	2.7.1.148	$1e^{-102}$	*Planktothrix agardhii* (94%)	-
150	NS500123:11:H0E9PAGXX:3:21403:9736:1272.1	4-hydroxy-3-methylbut-2-enyl diphosphate reductase	1.17.1.4	$4e^{-71}$	*Planktothrix agardhii* (100%)	-
139	NS500123:11:H0E9PAGXX:1:21204:9083:6857.2	isopentenyl pyrophosphate isomerase	5.3.3.2	$3e^{-57}$	*Planktothrix agardhii* (96%)	-
113	NS500123:11:H0E9PAGXX:2:11208:23616:6077	farnesyl-diphosphate synthase	2.5.1.10	$1e^{-50}$	*Planktothrix agardhii* (100%)	-

APPENDIX I - T&O-degrading bacteria abundances during each individual sampling date and phase of the reservoir

	Phase I			Phase II					Phase III		
Arthrobacter spp.	**5/15/13**	**5/23/13**	**6/11/13**	**6/27/13**	**7/11/13**	**7/25/13**	**8/6/13**	**8/22/13**	**9/4/13**	**10/1/13**	**10/23/13**
A. creatinolyticus	0.00000	0.00000	0.00004	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
A. psychrochitiniphilus	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
A. uratoxydans	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Bacillus spp.											
B. arbutinivorans	0.00009	0.00000	0.00003	0.00000	0.00000	0.00000	0.00002	0.00003	0.00005	0.00006	0.00010
B. aryabhattai	0.00000	0.00004	0.00000	0.00000	0.00000	0.00000	0.00002	0.00000	0.00001	0.00001	0.00000
B. beringensis	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002	0.00000	0.00000	0.00001	0.00002	0.00000
B. boroniphilus	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001
B. butanolivorans	0.00000	0.00000	0.00005	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002
B. cereus	0.00000	0.00000	0.00000	0.00003	0.00002	0.00008	0.00000	0.00002	0.00001	0.00000	0.00001
B. foraminis	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001	0.00001
B. funiculus	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
B. ginsengihumi	0.00000	0.00000	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
B. herbersteinensis	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002
B. horneckiae	0.00000	0.00000	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002
B. humi	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001	0.00000
B. litoralis	0.00000	0.00000	0.00005	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
B. longiquaesitum	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00004	0.00000	0.00001	0.00002	0.00004
B. nealsonii	0.00000	0.00000	0.00000	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
B. niacini	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00002	0.00005
B. olivae	0.00000	0.00001	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
B. oryzae	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
B. shandongensis	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
B. simplex	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00002	0.00000
B. siralis	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002	0.00003	0.00001
B. soli	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001	0.00000
B. thermoamylovorans	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
B. thioparans	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00003
Comamonas spp.											
C. composti	0.00000	0.00000	0.00004	0.00000	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
C. kerstersii	0.00025	0.00047	0.00018	0.00006	0.00010	0.00006	0.00008	0.00004	0.00003	0.00000	0.00003
C. koreensis	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
C. nitrativorans	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001	0.00000
C. odontotermitis	0.00001	0.00011	0.00065	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002	0.00003	0.00000
C. testosteroni	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000	0.00002	0.00000	0.00000	0.00000	0.00000
Enterobacter spp.											
E. amnigenus	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
E. hormaechei	0.00000	0.00000	0.00004	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

E. soli	0.00000	0.00001	0.00002	0.00001	0.00000	0.00000	0.00002	0.00000	0.00000	0.00000	0.00001
Flavobacterium spp.											
F. algicola	0.00006	0.00035	0.00010	0.00002	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. antarcticum	0.00021	0.00014	0.00009	0.00010	0.00005	0.00000	0.00008	0.00002	0.00001	0.00001	0.00004
F. aquatile	0.00087	0.00102	0.00011	0.00021	0.00001	0.00000	0.00000	0.00002	0.00001	0.00000	0.00003
F. branchiophilum	0.00040	0.00027	0.00022	0.00002	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001
F. cauense	0.00000	0.00002	0.00000	0.00000	0.00005	0.00018	0.00002	0.00005	0.00002	0.00000	0.00000
F. cheniae	0.00028	0.00015	0.00014	0.00006	0.00011	0.00000	0.00000	0.00000	0.00000	0.00000	0.00010
F. chungangense	0.00000	0.00007	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001
F. columnare	0.00001	0.00002	0.00000	0.00022	0.00003	0.00002	0.00000	0.00000	0.00001	0.00001	0.00000
F. croceum	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001	0.00000
F. defluvii	0.00001	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. flevense	0.00003	0.00007	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. frigidarium	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001
F. frigidimaris	0.00006	0.00004	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. gelidilacus	0.00026	0.00005	0.00017	0.00000	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00004
F. glaciei	0.00001	0.00003	0.00001	0.00000	0.00000	0.00000	0.00002	0.00000	0.00000	0.00000	0.00000
F. glycines	0.00004	0.00015	0.00009	0.00003	0.00000	0.00000	0.00000	0.00002	0.00001	0.00001	0.00004
F. granuli	0.00002	0.00007	0.00003	0.00002	0.00000	0.00000	0.00000	0.00000	0.00001	0.00002	0.00000
F. hydatis	0.00000	0.00051	0.00024	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. kamogawaensis	0.00046	0.00034	0.00008	0.00000	0.00001	0.00000	0.00000	0.00001	0.00002	0.00002	0.00000
F. micromati	0.00000	0.00021	0.00004	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. omnivorum	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. pectinovorum	0.00000	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. reichenbachii	0.00036	0.00022	0.00007	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. resistens	0.00042	0.00017	0.00003	0.00002	0.00000	0.00000	0.00000	0.00000	0.00002	0.00003	0.00000
F. saliperosum	0.00064	0.00054	0.00031	0.00002	0.00003	0.00000	0.00000	0.00002	0.00005	0.00006	0.00003
F. succinicans	0.00015	0.00094	0.00043	0.00007	0.00020	0.00000	0.00000	0.00002	0.00005	0.00002	0.00005
F. suncheonense	0.00024	0.00018	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
F. swingsii	0.00012	0.00003	0.00000	0.00002	0.00010	0.00000	0.00000	0.00002	0.00002	0.00001	0.00000
F. terrigena	0.00175	0.00087	0.00041	0.00022	0.00002	0.00000	0.00004	0.00006	0.00006	0.00008	0.00029
F. weaverense	0.00050	0.00032	0.00012	0.00057	0.00023	0.00018	0.00014	0.00011	0.00009	0.00009	0.00087
F. xinjiangense	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Novosphingobium spp.											
N. acidiphilum	0.00002	0.00008	0.00013	0.00005	0.00010	0.00012	0.00008	0.00003	0.00005	0.00004	0.00005
N. aromaticivorans	0.00004	0.00000	0.00005	0.00008	0.00001	0.00002	0.00004	0.00000	0.00001	0.00001	0.00006
N. hassiacum	0.00001	0.00004	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001	0.00002
N. indicum	0.00006	0.00008	0.00004	0.00010	0.00000	0.00010	0.00000	0.00002	0.00001	0.00000	0.00006
N. lentum	0.00021	0.00016	0.00006	0.00006	0.00001	0.00000	0.00000	0.00000	0.00001	0.00001	0.00000
N. mathurense	0.00002	0.00007	0.00000	0.00004	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002
N. stygium	0.00041	0.00073	0.00019	0.00043	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
N. subterraneum	0.00000	0.00012	0.00022	0.00001	0.00000	0.00000	0.00000	0.00000	0.00001	0.00002	0.00000
N. taihuense	0.00000	0.00003	0.00013	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

N. yangbajingensis	0.00003	0.00004	0.00004	0.00005	0.00011	0.00030	0.00002	0.00006	0.00004	0.00002	0.00011
Pseudomonas spp.											
P. alcaligenes	0.00000	0.00000	0.00013	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. anguilliseptica	0.00048	0.00022	0.00016	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. benzenivorans	0.00000	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. borealis	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. brenneri	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001	0.00000
P. caricapapayae	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. chloritidismutans	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001
P. clemancea	0.00000	0.00001	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. coronafaciens	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. corrugata	0.00006	0.00005	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. cremoricolorata	0.00000	0.00004	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. entomophila	0.00000	0.00006	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. gessardii	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. guineae	0.00000	0.00001	0.00017	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. jessenii	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. lundensis	0.00004	0.00001	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. lutea	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. mandelii	0.00000	0.00006	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. migulae	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. moraviensis	0.00000	0.00009	0.00000	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. mosselii	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. mucidolens	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. otitidis	0.00000	0.00008	0.00002	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00005
P. panipatensis	0.00003	0.00001	0.00005	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. parafulva	0.00000	0.00000	0.00000	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. pavonaceae	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. plecoglossicida	0.00003	0.00025	0.00007	0.00002	0.00000	0.00000	0.00002	0.00000	0.00000	0.00000	0.00000
P. pseudoalcaligenes	0.00000	0.00000	0.00003	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. resinovorans	0.00000	0.00001	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. rhodesiae	0.00000	0.00004	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. savastanoi	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. taetrolens	0.00002	0.00000	0.00000	0.00000	0.00002	0.00002	0.00002	0.00000	0.00000	0.00000	0.00000
P. taiwanensis	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. teessidea	0.00001	0.00005	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. thermotolerans	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001	0.00001	0.00000
P. tremae	0.00000	0.00004	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. umsongensis	0.00001	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
P. vancouverensis	0.00000	0.00001	0.00001	0.00000	0.00000	0.00000	0.00002	0.00000	0.00000	0.00000	0.00000
Rhodococcus spp.											
R. imtechensis	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
R. kyotonensis	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000

R. percolatus	0.00030	0.00167	0.00075	0.00111	0.00101	0.00037	0.00024	0.00032	0.00031	0.00037	0.00048
R. yunnanensis	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Sphingomonas spp.											
S. asaccharolytica	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002	0.00000	0.00000	0.00000	0.00001
S. dokdonensis	0.00002	0.00000	0.00000	0.00000	0.00003	0.00000	0.00000	0.00000	0.00004	0.00000	0.00003
S. echinoides	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
S. elodea	0.00003	0.00001	0.00002	0.00002	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
S. fennica	0.00000	0.00001	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
S. ginsenosidimutans	0.00000	0.00001	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
S. hankookensis	0.00000	0.00004	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
S. hunanensis	0.00019	0.00025	0.00007	0.00002	0.00001	0.00002	0.00000	0.00004	0.00003	0.00003	0.00000
S. insulae	0.00000	0.00004	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
S. leidyia	0.00000	0.00000	0.00000	0.00000	0.00003	0.00000	0.00000	0.00000	0.00002	0.00000	0.00001
S. mali	0.00000	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
S. melonis	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00001
S. oligophenolica	0.00005	0.00008	0.00006	0.00004	0.00000	0.00004	0.00004	0.00003	0.00004	0.00006	0.00005
S. panni	0.00001	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
S. sanxanigenens	0.00000	0.00010	0.00010	0.00003	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00008
S. soli	0.00000	0.00004	0.00003	0.00000	0.00000	0.00000	0.00000	0.00002	0.00004	0.00003	0.00000
S. wittichii	0.00004	0.00027	0.00011	0.00004	0.00002	0.00000	0.00000	0.00000	0.00000	0.00000	0.00029
S. yunnanensis	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00002	0.00001	0.00000	0.00000
Sphingopyxis spp.											
Sp. alaskensis	0.00000	0.00000	0.00000	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Sp. chilensis	0.00000	0.00000	0.00000	0.00003	0.00001	0.00000	0.00000	0.00000	0.00002	0.00002	0.00000
Sp. granuli	0.00000	0.00002	0.00000	0.00001	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000	0.00000
Sp. witflariensis	0.00000	0.00005	0.00007	0.00013	0.00001	0.00002	0.00000	0.00002	0.00002	0.00003	0.00001

APPENDIX II – Spearman's *rho* (r_s) correlations of identified T&O-degrading bacteria with total methylisoborneol (MIB), total geosmin (GSM) concentrations and their Bound to Dissolved (B:D) ratios in Eagle Creek Reservoir.

⇨ Correlations with strong statistical significance are in **bold** with $p < 0.05$, *$p < 0.01$ and **$p < 0.001$.

Actinobacteria (→ GSM)	MIB	B:D	GSM	B:D
Arthrobacter creatinolyticus	0.28	-0.06	-0.24	**-0.68**
Arthrobacter psychrochitiniphilus	0.10	-0.10	0.00	-0.10
Arthrobacter uratoxydans	0.30	-0.36	0.10	-0.45
Rhodococcus imtechensis	0.30	-0.36	0.10	-0.45
Rhodococcus kyotonensis	0.50	0.51	0.50	0.40
Rhodococcus percolatus	0.38	0.07	0.11	-0.24
Rhodococcus yunnanensis	0.30	-0.36	0.10	-0.45

Bacteroidetes (→ MIB)	MIB	B:D	GSM	B:D
Flavobacterium algicola	**0.60**	0.32	0.36	-0.24
Flavobacterium antarcticum	0.33	0.46	0.21	0.00
Flavobacterium aquatile	0.52	0.49	0.43	0.12
Flavobacterium branchiophilum	0.58	0.15	0.51	-0.12
Flavobacterium cauense	-0.51	-0.12	-0.30	0.21
Flavobacterium cheniae	0.57	0.08	0.59	-0.03
Flavobacterium chungangense	0.48	0.30	0.23	-0.02
Flavobacterium columnare	0.25	0.30	-0.05	-0.37
Flavobacterium croceum	0.46	0.17	0.43	-0.10
Flavobacterium defluvii	0.13	0.47	-0.13	-0.30
Flavobacterium flevense	**0.66**	0.33	**0.68**	0.13
Flavobacterium frigidarium	-0.10	-0.36	0.30	0.50
Flavobacterium frigidimaris	0.50	0.51	**0.66**	0.35
Flavobacterium gelidilacus	0.50	-0.08	**0.70**	0.12
Flavobacterium glaciei	0.28	0.33	0.37	0.28
Flavobacterium glycines	**0.66**	0.39	**0.61**	0.18
Flavobacterium granuli	**0.86****	0.49	0.43	-0.31
Flavobacterium hydatis	0.57	0.37	0.18	-0.22
Flavobacterium kamogawaensis	**0.78***	0.27	**0.69**	-0.09
Flavobacterium micromati	**0.61**	0.17	0.47	0.02
Flavobacterium omnivorum	0.20	0.20	0.40	0.10
Flavobacterium pectinovorum	0.50	0.51	0.50	0.40
Flavobacterium reichenbachii	**0.62**	0.46	0.45	-0.10
Flavobacterium resistens	**0.89****	0.51	**0.61**	-0.19
Flavobacterium saliperosum	**0.85****	0.20	**0.80***	-0.04
Flavobacterium succinicans	**0.62**	0.17	0.41	-0.21
Flavobacterium suncheonense	**0.62**	0.29	**0.67**	0.10
Flavobacterium swingsii	0.33	0.51	0.17	-0.11
Flavobacterium terrigena	**0.70**	0.44	**0.65**	0.16

Flavobacterium weaverense	-0.01	0.09	0.16	0.24
Flavobacterium xinjiangense	0.50	0.51	0.50	0.40

Firmicutes (→ MIB)	**MIB**	**B:D**	**GSM**	**B:D**
Bacillus arbutinivorans	0.17	-0.12	0.45	0.32
Bacillus aryabhattai	0.31	0.39	0.22	0.27
Bacillus beringensis	0.16	-0.20	0.09	-0.20
Bacillus boroniphilus	-0.10	-0.36	0.30	0.50
Bacillus butanolivorans	0.17	-0.20	-0.09	-0.37
Bacillus cereus	-0.54	-0.22	-0.55	-0.17
Bacillus foraminis	0.29	-0.20	0.34	0.12
Bacillus funiculus	0.30	-0.36	0.10	-0.45
Bacillus ginsengihumi	0.30	-0.36	0.10	-0.45
Bacillus herbersteinensis	-0.10	-0.36	0.30	0.50
Bacillus horneckiae	0.08	-0.20	-0.04	-0.14
Bacillus humi	0.39	0.01	0.16	-0.23
Bacillus litoralis	0.30	-0.36	0.10	-0.45
Bacillus longiquaesitum	-0.07	-0.35	0.04	0.16
Bacillus nealsonii	-0.16	-0.01	-0.50	-0.55
Bacillus niacini	0.50	0.01	**0.70**	0.37
Bacillus olivae	0.34	**0.68**	-0.07	-0.09
Bacillus oryzae	0.10	-0.10	0.00	-0.10
Bacillus shandongensis	0.50	0.51	0.50	0.40
Bacillus simplex	0.39	0.01	0.16	-0.23
Bacillus siralis	0.31	-0.17	0.31	0.05
Bacillus soli	**0.68**	0.40	0.50	0.12
Bacillus thermoamylovorans	0.10	-0.10	0.00	-0.10
Bacillus thioparans	-0.01	-0.36	0.24	0.34

β-Proteobacteria (→ GSM)	**MIB**	**B:D**	**GSM**	**B:D**
Comamonas composti	0.11	-0.53	-0.05	-0.57
Comamonas kerstersii	0.23	0.23	0.19	-0.06
Comamonas koreensis	0.20	0.20	0.40	0.10
Comamonas nitrativorans	0.54	-0.20	0.21	-0.49
Comamonas odontotermitis	**0.88****	0.14	**0.64**	-0.20
Comamonas testosteroni	-0.40	0.28	**-0.66**	-0.14

α-Proteobacteria (→ GSM)	**MIB**	**B:D**	**GSM**	**B:D**
Novosphingobium acidiphilum	-0.03	-0.46	-0.17	-0.38

Novosphingobium aromaticivorans	-0.09	-0.30	-0.15	-0.31
Novosphingobium hassiacum	**0.63**	0.27	**0.87****	0.55
Novosphingobium indicum	0.16	0.23	0.14	0.11
Novosphingobium lentum	**0.73**	0.44	0.40	-0.36
Novosphingobium mathurense	0.43	0.59	0.39	0.27
Novosphingobium stygium	0.55	0.47	0.24	-0.24
Novosphingobium subterraneum	**0.81***	0.22	0.33	-0.39
Novosphingobium taihuense	0.54	0.27	0.13	-0.32
Novosphingobium yangbajingensis	-0.40	-0.35	-0.15	0.16
Sphingomonas asaccharolytica	-0.25	-0.10	0.12	0.43
Sphingomonas dokdonensis	-0.02	-0.40	0.20	0.04
Sphingomonas echinoides	0.50	0.51	0.50	0.40
Sphingomonas elodea	0.45	0.22	0.12	-0.50
Sphingomonas fennica	**0.61**	0.17	0.47	0.02
Sphingomonas ginsenosidimutans	0.34	**0.68**	-0.07	-0.09
Sphingomonas hankookensis	0.50	0.51	0.50	0.40
Sphingomonas hunanensis	**0.70**	0.55	0.52	-0.03
Sphingomonas insulae	0.50	0.51	0.50	0.40
Sphingomonas leidyia	-0.14	-0.52	0.01	-0.03
Sphingomonas mali	0.50	0.51	0.50	0.40
Sphingomonas melonis	-0.10	-0.36	0.30	0.50
Sphingomonas oligophenolica	**0.86****	0.24	**0.76***	0.12
Sphingomonas panni	-0.03	-0.15	0.11	-0.18
Sphingomonas sanxanigenens	0.48	0.04	0.34	-0.01
Sphingomonas soli	**0.65**	0.32	0.42	0.03
Sphingomonas wittichii	0.49	0.01	0.49	0.09
Sphingomonas yunnanensis	-0.26	0.18	-0.24	0.18
Sphingopyxis alaskensis	-0.20	-0.36	-0.20	-0.30
Sphingopyxis chilensis	0.19	0.14	-0.35	**-0.63**
Sphingopyxis granuli	0.40	**0.69**	0.07	0.02
Sphingopyxis witflariensis	0.51	0.23	-0.05	-0.53

γ-Proteobacteria (→ MIB)	**MIB**	**B:D**	**GSM**	**B:D**
Enterobacter amnigenus	0.00	0.41	-0.50	-0.45
Enterobacter hormaechei	0.30	-0.36	0.10	-0.45

Enterobacter soli	0.01	0.10	-0.18	-0.03
Pseudomonas alcaligenes	0.30	-0.36	0.10	-0.45
Pseudomonas anguilliseptica	**0.62**	0.46	0.45	-0.10
Pseudomonas benzenivorans	0.50	0.51	0.50	0.40
Pseudomonas borealis	0.50	0.51	0.50	0.40
Pseudomonas brenneri	0.39	0.01	0.16	-0.23
Pseudomonas caricapapayae	0.50	0.51	0.50	0.40
Pseudomonas chloritidismutans	-0.10	-0.36	0.30	0.50
Pseudomonas clemancea	0.34	**0.68**	-0.07	-0.09
Pseudomonas coronafaciens	0.20	0.20	0.40	0.10
Pseudomonas corrugata	**0.62**	0.29	**0.67**	0.10
Pseudomonas cremoricolorata	**0.61**	0.17	0.47	0.02
Pseudomonas entomophila	0.50	0.51	0.50	0.40
Pseudomonas gessardii	0.50	0.51	0.50	0.40
Pseudomonas guineae	0.58	0.06	0.42	-0.09
Pseudomonas jessenii	0.00	0.41	-0.50	-0.45
Pseudomonas lundensis	**0.60**	0.19	**0.62**	0.00
Pseudomonas lutea	0.30	-0.36	0.10	-0.45
Pseudomonas mandelii	0.40	**0.69**	0.07	0.02
Pseudomonas migulae	0.20	0.20	0.40	0.10
Pseudomonas moraviensis	0.43	0.59	0.06	-0.04
Pseudomonas mosselii	0.50	0.51	0.50	0.40
Pseudomonas mucidolens	0.50	0.51	0.50	0.40
Pseudomonas otitidis	0.42	-0.24	0.53	0.21
Pseudomonas panipatensis	**0.61**	0.13	0.59	-0.07
Pseudomonas parafulva	0.00	0.41	-0.50	-0.45
Pseudomonas pavonaceae	0.30	-0.36	0.10	-0.45
Pseudomonas plecoglossicida	0.50	0.44	0.29	-0.07
Pseudomonas pseudoalcaligenes	0.24	-0.01	-0.26	**-0.67**
Pseudomonas resinovorans	**0.60**	0.00	0.39	-0.14
Pseudomonas rhodesiae	0.50	0.51	0.50	0.40
Pseudomonas savastanoi	0.30	-0.36	0.10	-0.45
Pseudomonas taetrolens	-0.39	-0.33	-0.15	-0.08
Pseudomonas taiwanensis	0.50	0.51	0.50	0.40
Pseudomonas teessidea	**0.69**	0.27	**0.67**	0.08
Pseudomonas thermotolerans	0.39	0.01	0.16	-0.23
Pseudomonas tremae	**0.61**	0.17	0.47	0.02
Pseudomonas umsongensis	0.54	0.55	**0.67**	0.39
Pseudomonas vancouverensis	0.10	0.14	0.07	0.17

CURRICULUM VITAE
NICOLAS ANDRE CLERCIN

Research interests

Water quality, limnology, eutrophication, biodiversity, microbial ecology, phytoplankton, biogeochemistry, ecotoxicology, eDNA, metabarcoding, remote sensing and public health.

Work experience

- Research Scientist at the **CENTER FOR EARTH AND ENVIRONMENTAL SCIENCE,** *2008-2016, Indianapolis, IN, USA*
- Hydro-biologist at **AQUASCOP,** 2007, *Angers, France*
- R&D Engineer at **ANJOU RECHERCHE – VEOLIA EAU,** 2005, *Paris, France*
- Assistant Engineer at **UNIVERSITY OF RENNES 1,** 2004, *France*
- Intern at **UNIVERSITY OF LYON,** 2004, *France*
- Intern at **INRA LAKE RESEARCH STATION,** 2004, *Thonon-les-Bains, France*
- Lab Analyst at **UNIVERSITY OF RENNES 1,** 2003, *France*

Education

2019 **PhD,** *Indiana University – Purdue University, Indianapolis (IUPUI)*
Major in Applied Earth Sciences (Advisor: Dr. Gregory Druschel)
Minor in Public Health (Advisor: Dr. Max Jacobo Moreno-Madriñán)

2005 **M. Sc. in Ecology, Water and Soils,** *University of Rennes I, France*

2003 **Maîtrise in Population Biology and Ecosystems,** *University of Rennes I*

2002 **B. Sc. in Biology of Organisms,** *University of Rennes I*

Professional affiliations

- American Water Resources Association (AWRA)
- Indiana Lakes Management Society (ILMS)
- North American Lake Management Society (NALMS)
- Groups of scientific interest on Cyanobacteria

Conference talks during PhD course

1. **Clercin, N.,** Druschel, G. – Influence of environmental factors on off-flavor metabolite production by bacteria in a eutrophic reservoir. 38th North American Lakes Managers Society (*NALMS*), Annual Symposium, Cincinnati, OH, November 2018.
2. **Clercin, N.** – *Communautés bactériennes et composés organiques volatiles dans un réservoir eutrophe.* IRSTEA, Bordeaux, March 2016.
3. **Clercin, N.** – *Facteurs Environnementaux et Origine des Composés Goût et Odeur, MIB et Géosmine, dans un Réservoir Eutrophe.* Group of scientific interest on Cyanobacteria, Annual Colloquium, Bordeaux, March 2016.
4. **Clercin, N.** – *Eagle Creek Reservoir – 2009-2015.* Citizens Water TAG, Indianapolis, IN, December 2015.
5. **Clercin, N.** – *Characterization of Taste-and-Odor-Producing and –Degrading Bacteria in a Central Indiana Reservoir.* 35th NALMS Annual Symposium, Saratoga Springs, NY, November 2015.
6. **Clercin, N.** – *Taste-and-Odor Occurrences in Central Indiana Reservoirs.* 36th Annual Indiana Water Resources Association Conference. Muncie, IN, June 2015.
7. Harris, T. D., Smith, V. H., Graham, J. L., Van de Waal, D. B., Tedesco, L. P. and **Clercin, N.** – *Combined Effects of the Nitrogen to Phosphorus Ratio and Nitrogen Speciation on Three Cyanobacterial Metabolite Concentrations in Eutrophic Reservoirs.* ASLO, Aquatic Sciences Meeting, Granada, Spain, February 2015.
8. **Clercin, N.** – *Taste-and-Odor Occurrences in Central Indiana Reservoirs – Data Summary 2009-2014.* Citizens Water TAG, Indianapolis, IN, January 2015.
9. **Clercin, N.,** Druschel, G. – *Identification of Taste-and-Odor Producing and Degrading Bacteria in a Freshwater Reservoir, Central Indiana.* 3rd Midwest Geobiology Symposium. Field Museum, Chicago, IL, September 2014.
10. **Clercin, N.** – *Using Metagenomics Tools to Identify Taste-and-Odor -Producing and -Degrading Bacteria in Eagle Creek Reservoir, Central Indiana.* Illumina Discovery Symposium, Boston, MA, May 2014.
11. **Clercin, N.** – *Potential Triggers for Algal Growth and Metabolites Production in Eagle Creek Reservoir, Central Indiana.* Urban Health Conference, Indianapolis, IN, April 2014.

12. **Clercin, N.** – *2009-2013 Data Analysis of Taste-and-Odor Compounds in Eagle Creek Reservoir*. Citizens Water TAG, Indianapolis, December 2013.
13. Tedesco, L.P., Graham, J.L., **Clercin, N.,** and Stouder, M. *Cyanobacterial Assemblages and Environmental Variables Associated with the Co-Occurrence of Cyanotoxins and Taste-and-Odor Compounds in the Midwestern United States*. 9th International Conference on Toxic Cyanobacteria, Johannesburg, South Africa, August 2013.
14. **Clercin, N.** – *Cyanobacteria and Algal Metabolites in Central Indiana Drinking Water Supply Reservoirs.* Scientech Club, Indianapolis, IN, July 2013.
15. **Clercin, N.** – *Cyanobacterial Production of Taste-and-Odor Compounds in Central Indiana Drinking Water Supply Reservoirs.* Argonne National Lab, Chicago, IL, June 2013.
16. **Clercin, N.** – *Taste-and-Odor Occurrences Associated with Resident Cyanobacteria in a Central Indiana Drinking Water Supply Reservoir*. 25th Annual Indiana Lakes Management Society Conference (ILMS). Angola, IN, March 2013.
17. Tedesco, L.P., Graham, J.L., **Clercin, N.**, and Stouder, M. – *Cyanobacterial Assemblages and Environmental Variables Associated with the Co-Occurrence of Cyanotoxins and Taste-and-Odor Compounds in Midwestern Drinking-Water Supply Reservoirs*, 2013 ASLO, Aquatic Sciences Meeting, New Orleans, LA, February, 2013.

Poster presentations during PhD course

- Druschel, G., Kafantaris, F-C. A., Schroth, A.W., Fike, D. A., Orphan, V. J., Schmitt-Kopplin, P., Dvorski, S. and **Clercin, N.** – *What greater spatial and temporal geochemistry detail can add to geobiology?* AGU Fall Meeting, San Francisco, CA, December 2016.
- **Clercin, N.,** Druschel, G. – *Environmental Factors Promoting the Growth of Taste-and-Odor Producing Cyanobacteria in a Eutrophic Resevoir, Central Indiana.* 4th Midwest Geobiology Symposium. Bloomington, IN, September 2015.
- **Clercin, N.,** Druschel, G. – *Potential Triggers for Algal Growth and Metabolites Production in Eagle Creek Reservoir, Central Indiana. Vermont EPSCoR Annual State Meeting.* Burlington, VT, August 2014.

- Van Hove, S., Teiling, C., Schmieder, R., Chorny, I., Steffy, B., **Clercin, N.,** Stouder, M., Gray, M. – *A Metagenomic Study of a Drinking Water Supply Reservoir in Central Indiana.* Illumina Discovery Symposium, Boston, MA, May 2014.
- **Clercin, N.**, Druschel, G. – *Potential Triggers for Algal Growth and Metabolites Production in Eagle Creek Reservoir, Central Indiana*. Crossroads Geology Conference. Bloomington, IN, March 2014.
- **Clercin, N.**, Druschel, G. – *Seasonal Occurrences and Production of Odorous Algal Metabolites (MIB and Geosmin) in Eagle Creek Reservoir, Central Indiana*. 2nd Midwest Geobiology Symposium. Indianapolis, IN, September 2013.

Publications

- **Clercin, N**., Druschel, G.K. 2019. Influence of environmental factors on off-flavor metabolite production by bacteria in a eutrophic reservoir. Submitted to *Water Resources Research*.
- Harris, T.D., Graham, J.L., Van de Waal, D.B., Smith, V.H., Tedesco, L.P., **Clercin, N.** 2016. Combined effects of nitrogen to phosphorus ratios and nitrogen speciation on cyanobacterial metabolite concentrations in eutrophic Midwestern USA reservoirs. *Inland Waters*, 6(2): 199-210
- Song, K.S., Li, L., Tedesco, L.P., **Clercin, N.,** Li, L., Shi, K. 2014. Spectral characterization of colored dissolved organic matter for productive inland waters and its source analysis. *Chinese Geographical Science.* 6: 1-14.
- Song, K. S., Li, L., Tedesco, L.P., **Clercin, N.,** Hall, B., Li., S., Shi, K., Liu, D., Sun, Y. 2013. Remote estimation of phycocyanin (PC) for inland waters coupled with YSI PC fluorescence probe. *Environmental Science and Pollution Research*, 20(8): 5330-40.
- Song, K. S., Li, L., Tedesco, L.P., Li, S., **Clercin, N.A.,** Hall, B.E., Li, Z., Shi, K. 2012. Hyperspectral Determination of Eutrophication for a Water Supply Source via Genetic Algorithm-Partial Least Squares (GA-PLS) modeling. *Science of the Total Environment*, 426: 220-232.

Technical reports

- Druschel, G., **Clercin, N.** 2016. Seasonal Occurrences, Production, and Biodegradation of Algal Metabolites (MIB, Geosmin, and Microcystin) in Eagle Creek Reservoir, a Drinking Water Supply Reservoir in Central Indiana. 14 pp.
- **Clercin, N.** 2013. Algal Ecology, Taste and Odor, and Microcystin Occurrence in Patoka Lake, Southern Indiana. 21 pp.
- **Clercin, N.** 2012. Algal Ecology, Taste and Odor, and Microcystin Occurrence in Patoka Lake, Southern Indiana. 49 pp.
- **Clercin, N.,** Stouder, M., Tedesco, L.P. 2012. Cyanobacteria and Microcystin Occurrence in Indiana. 2011 Final Report. 26 pp.
- Tedesco, L.P., **Clercin, N.** 2011. Final Report – Algal Ecology, Taste and Odor, and Microcystin Occurrence in Patoka Lake, Southern Indiana. 42 pp.
- Tedesco L, **Clercin N.** 2011. Algal Ecology, Cyanobacteria Toxicity and Secondary Metabolites Production of the Three Eutrophic Drinking Water Supply and Recreational Use Reservoirs in Central Indiana. 2010 Research Project Final Report. 25-29 pp.
- Tedesco, L.P., **Clercin, N.** 2010. Final Report – Algal Ecology and Algal Metabolites Occurrences in Patoka Lake, Southern Indiana. 31 pp.