Biochemical characterization of particulate and dissolved organic matter in pre-alpine Lake Lunz

by

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April 2016

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Thesis submitted for the award of the title

"Master of Science"

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This thesis is submitted in partial fulfillment of the requirements of international academic degree of
Master of Science in Limnology and Wetland Management
jointly awarded by
University of Natural Resources and Life Sciences (BOKU), Vienna, Austria
UNESCO-IHE Institute for Water Education, Delft, the Netherlands
Egerton University, Njoro, Kenya

University of Natural Resources and Life Sciences (BOKU), Vienna, Austria

April 2016

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Acknowledgements

Today, I am proudly concluding my master thesis and of course do feel elated and complacent with the achievement but I was not alone in this arduous journey and could have not succeeded without the support of several.

Firstly, I would like to express my sincere gratitude to my supervisor Dr. Martin Kainz for his keen involvement in overall process of designing and implementation of the research project and writing my thesis and even beyond. His dignified demeanor and consistent encouragement provided me a conducive working atmosphere, and hence was an impetus to summit the goal. He has been motivating, enlightening and supportive in all walks of life during my stay in Lunz and provided always an insight and direction-right up to the end of journey. I can't thank him enough but I will remain grateful and owe him my success.

Furthermore, I would like to extend my thankfulness to Dr. Thomas Hein for introducing me to the WasserCluster, Lunz and most importantly for being a generous co-supervisor and extremely supportive in sorting out the academic and institutional arrangement involved in the entire process. Also I like to thank Dr. Elisabet Ejarque, Katharina Winter, Stefanie Danner and Gertraud Steniczka who always shared their precious time during the entire process of field and lab work and more importantly teaching me the laboratory analytical techniques. Elisabet (Bet) and Katharina (Kathi), were the first who welcomed me with their radiant smiles and evergreen friendly gestures, and have always been of tremendous help no matter what. Bet, thank you for being my "friend with solitude". I also want to specially thank Mrs. Josefa Sommer and Ms. Eva Lugbauer for all sort of logistic and administrative arrangement and I may simply state that they are perfect. I must mention another special and charismatic friend Marina Jecmenica who shared with me the lonely autumn/winter time in Lunz and enriched me with many precious memories though I persistently kept annoying her by calling a freak. Thank you Marina for being there and you shall be remembered, forever. I can't list out individual names of my BOKU and LWM friends, and WasserCluster colleagues but indeed I owe them a big thank for making my stay in Austria memorable.

The study involved courses at various universities located in different countries and would have never been possible without the financial support of Nuffic under the Netherlands Fellowship Programme (NFP) and partner universities. Particularly, I would like to thank Mr. Gerold Winkler, Manager-IPGL, BOKU, Austria, Prof. Nzula Kitaka, Egerton University Kenya and Edwin Hes, coordinator, UNESCO-IHE, the Netherlands for taking

care of all the involved academic and administrative matters. I must admire the IPGL staff, Mr Gerold Winkler, Hanna Hentschel (Ex-employee) and Nina Haslinger for always being flexible, generous and accommodative. Field expeditions and analytical laboratory work associated with the research project were supported by WasserCluster Lunz and the research project EXCARB funded by the Austrian Academy of Sciences.

Of course no acknowledgments would be complete without expressing my profound gratitude to my parents and brothers for providing me with continuous support and encouragement. Mum and dad, I have always lived with your taught lessons of believing in self, tolerance, resilience, love for nature, and most importantly to be a compassionate human being. My brothers Wajeeh Ullah, Fazal Wadood, Zia Ullah and Adnan Saeed have always been my true friends and supporters. Thank you all for always being there.

In addition, the acknowledgments would not be complete without mentioning my daughters, four year-old Amna Khan (Ammo) and a year and a half-old Fatima Khan. They both have been my rays of hope, twinkle in my eye and to be honest reason of living. Ammo has always been telling me that she was my lonely angel and I only belong to her, but truly she is and I do. Amna and Fatima, I am proud of you and life without you would not have been the same and so meaningful.

Last, but certainly not least, I must acknowledge with tremendous gratitude my soul mate and partner Seema Khan. Her love and company has always been my strength and an essential fuel for moving on. She remained very patient with my long absentee and proved herself a wonderful mother and strong women by understanding the essence of time, coping with and facing-up the situations. I want her to know that I do much care for her and will always remain indebted for loving and supporting me. Seema, without you, I never would have known the feeling of being loved with complete intimacy and I rambled a lot but could not find the right words that can express my feeling for you and that what your presence in my life really meant to me.

Sami

March 31, 2016

Abstract

In this field study, we investigated how particulate organic matter (POM) and dissolved organic matter (DOM) quantity and its biochemical quality changed between lake inflow and outflow as well as within the oligotrophic, pre-alpine Lake Lunz, Austria, from 2013 to 2015. We tested the hypothesis that irrespective of seasons, stream water recharging the lake contains predominantly recalcitrant POM (>1.2 µm particle size) and DOM (<0.2 µm), whereas outflowing lake water is mostly composed of more labile, algae-derived organic matter. Samples were collected at a monthly basis from the lake layers, inflowing and outflowing streams, and analysed for fatty acids as biochemical indicators of POM and optical indices and matrices for DOM quality. Results showed that increasing precipitation and runoff predicted significantly increasing inflowing concentrations of POM ($r = 0.72$, $R^2=0.52$, *Sig. F*<0.001) and DOC ($r = 0.40$, $R^2=0.16$, *Sig. F* =0.04). The lake retained ~58% of total imported POM, but exported ~3X, ~8X, and ~6X more bacterial fatty acids (BAFA), and algae-derived omega-3 PUFA and omega-6 PUFA, respectively, than the inflow. Long-chain saturated fatty acids (used as proxy for terrestrial organic matter) constituted ~9% in inflow and ~6% of total SAFA in the outflow. The optical characterization of DOM, as indicated by qualitative fluorophores, depicted that the prevalence of allochthonous DOM in inflow and hypolimnion was significantly higher than in the epilimnion and outflow (p<0.05). The values of the humification (HIX) and biological indexes (BIX) also suggest that the inflow stream was a predominant source of terrigenous organic matter, whereas the epilimnion and outflow indicated a prevalence of high bacterial and algal derived DOM. In general, Lake Lunz exports on average 8X more labile POM (algae-derived) and DOM containing 21% less allochthonous and 35% more autochthonous organic matter than the inflow. These results suggest that the oligotrophic, pre-alpine Lake Lunz is a biochemical upgrader within the fluvial network of this drainage basin and supplies highly labile and nutritional POM and DOM to consumers further downstream, irrespective of the season.

Chapter 1. Introduction

1.1 Background

Lakes are not isolated water bodies, but part of a larger complex landscape system that includes watershed and associated surface and ground water networks (Holdren *et al.*, 2001). Landscape functions may be seen as analogous to a funnel where lakes act as bottom funnel and receive organic matter, nutrients and energy through runoff and base flow (Prairie, 2008). Being a receiver, reflector, and supplier to downstream ecosystems, lakes are an integral part of the landscape and thus play a vital role in biogeochemical cycling of organic matter (Holdren *et al.*, 2001; Wetzel, 2001).

Organic matter (OM) refers to organic molecules that predominantly comprise carbon, oxygen and hydrogen along with a relative small fraction of nitrogen, phosphorous and sulphur. The complex composition of OM makes it difficult to analytically quantify and therefore a universal understanding has been developed to use the measurement of organic carbon (OC) as proxy of OM (Kainz, 1997). The fractionation criteria of dissolved and particulate OM is still debated and ranges from OM <0.5 µm (Wetzel, 1983), <0.45 µm, (Lock *et al.*, 1977) or 0.2 µm (Kainz, 1997; Sundh, 1992) that is considered as dissolved OM (DOM). When using DOM <0.2 µm it is argued that DOM is free of bacteria that are typically >0.2 µm.

The amount of particulate OM (POM) is very low relative to DOM in aquatic ecosystems. Within the total organic matter, DOM constitutes approx. 90% (Thurman, 1985). Prairie (2008) questioned the OM constitution in lake water and highlighted that, with the exception of deep settled carbon in the sediments, DOM comprises the largest reservoir of organic carbon in the water column, whereas POM, including heterotrophic bacterial biomass and the combined biomass of phytoplankton, zooplankton, and fish, constitutes only about 2% of the total carbon mass.

Total OM in lakes is a heterogeneous mixture of materials originating from the degradation of allochthonous and autochthonous vegetation, animals and microorganisms (Huguet *et al.*, 2010): Autochthonous DOM is produced within aquatic systems, mostly due to algal exudates and decaying of organisms (Meili, 1992; Wetzel, 1983). The concentration and chemical composition of OM in lakes vary with seasons due inflow water volume, photo and microbial degradation, and autochthonous production (Mostofa *et al.*, 2015). DOM composition also varies due to size and catchment types as mostly intermediate order and

pristine streams are transporting up to 99% allochthonous OM (Wetzel, 2001). Qualitative characteristics and structural composition of OM also depend on terrestrial and aquatic sources, mostly due to differences in OM synthesis (Kirchman, 2012; Meyers & Eadie, 1993).

Biochemical assessments are required to characterize OM quality, its diagenesis and cycle in lakes. There are basically two complementary approaches for analyzing the composition of POM and DOM:

a) molecular analysis of POM, including major biochemical compounds (protein, carbohydrates and lipids) and,

b) high resolution fluorescence spectroscopy of DOM. The biochemical and optical characterization of OM is also important for knowing the environmental stability (recalcitrance) and degradability (lability). Microbial communities (bacteria, protists and metazoans) in lakes decompose the biodegradable fraction of POM and DOM (Meyers & Ishiwatari, 1993). According to Findlay (2002), DOM as being the largest bioavailable source of carbon affect a large number of aquatic ecosystem processes and functioning.

Algal exudates are a major source of labile carbon in lakes and favour high rates of respiration and assimilation by bacterial communities (Cole *et al.*, 1982; Kainz, 1997). Algal exudates in lakes primarily depend on primary productivity and also on zooplankton and other aquatic animal activities (Bowszys *et al.*, 2014; Wetzel, 1983). Several studies have demonstrated high bacterial biomass as a function of high algal biomass (Vadstein *et al.*, 1989). The in-lake autotrophic production contributes enormously in upgrading the overall OM quality that constitutes essential biochemical compounds such as lipids. Some lipids and their fatty acids (FA) are essential for consumers, but only synthesized by algae. The term 'essential fatty acids' refers to omega-3 and -6 fatty acids that cannot be synthesized by animals because of their lack of specific enzymes. Unlike algae as plants in general, animals lack the enzymes delta-12 and -15 desaturases that are required to synthesize omega-3 and -6 polyunsaturated fatty acids (PUFA) *de novo*, respectively (Cook *et al.*, 2004). Thus, such lipids are essential for consumers, they must be acquired by diet and subsequently strongly support aquatic food webs (Kainz *et al.*, 2004).

Lipids are highly useful biochemical markers for identifying the quality of OM (Harvey *et al.*, 1997) as they have rather stable nature in comparison to other biochemical counterparts i.e. carbohydrates and proteins (Harvey *et al.*, 1995). The labile and aromatic proportion of

POM and DOM can be assessed by their lipid content (Perdue, 1984) and fluorescent components (Huguet *et al.*, 2009; Weishaar *et al.*, 2003), respectively. Fatty acids (FA) are major parts of lipids and chemically contain carboxylic group with a long aliphatic chain, which is either saturated or unsaturated (IUPAC, 1997). The saturation and unsaturation nature of FA determines, in part, the nutritional quality of organic matter. Algae are the main source of unsaturated FA (Gladyshev *et al.*, 2013) and algal species dominance, to a higher extent, are controlled by the trophic status of lakes (Krienitz *et al.*, 2006). PUFA are an important source of dietary essential fatty acid [n-3] for many terrestrial organisms. The evidence is that PUFA concentrations are higher in aquatic insects/birds than in terrestrial insects/birds (Arts *et al.*, 2009). Terrestrial carnivorous species have shown a significant decrease in PUFA concentrations as a results of decreasing reliance on aquatic resources (Koussoroplis *et al.*, 2008). Higher plants are rich in long chain saturated (SAFA), mono-unsaturated (MUFA) (Arts *et al.*, 2009; Harwood, 1996), while algae are rich in PUFA (Leonarda *et al.*, 2004). The organic matter that enter a lake from terrestrial ecosystems is typically rich in long chain SAFA, but poor in n-3 PUFA (Mills *et al.*, 2001).

The aim of this study was to investigate how POM and DOM quantity and its biochemical quality change between lake inflow and outflow, and in the Lake Lunz across seasons from 2013 to 2015. Particular emphasis is thereby put on the lake as a 'reactor' of organic matter upgrading by the production of highly nutritious algal OM. This Lake Lunz, as the case for a large number of other lakes, receives high amounts of particulate and dissolved organic matter from the drainage basin (allochthonous sources) with temporal variation due to rainfall and extreme weather events (Battin, 1999; Bretschko, 1990). Therefore, the pattern and amount of precipitation, seasons and recently more often occurring extreme weather events indirectly affecting the organic matter supply to the lake. The inflowing volume not only alters the inputs of OM to the lake, but also determines the OM residence time and outflowing discharge (Holdren *et al.*, 2001). Goodman *et al.* (2011) assessed long-term temporal variability of DOM concentration in seven paired lake inflows and outflows and revealed that the lakes buffer DOM concentration and act as sink during high flow seasons and source during droughts.

As a measure of OM quality in POM, its FA are determined with special emphasis on PUFA (used as a proxy of algal sources), bacterial fatty acids, and long-chain saturated fatty acids (markers for terrestrial OM), to assess its distribution and biochemical quality of OM in the lake. High resolution fluorescence spectroscopy were used for characterizing the

allochthonous and autochthonous fraction of DOM. The spectroscopic signals were transformed into optics-based indices and matrices for DOM quality determination such as Humification Index (HIX; indicator of allochthonous DOM), Biological Index (BIX; indicator of algal exudate), and fluorescent components of matrices such as Peak A, C (Humic-like; recalcitrant substances) and Peak B and T (Protein-like; labile substances), and $SUVA_{254}$ (aromatic carbon).

Based on the above mentioned rationale, we tested the hypothesis that Lake Lunz biochemically upgrades POM and DOM quality mostly via algae production and algal exudates, respectively, which will consequently increase bacterial OM in the lake and its outflow relative to its inflow.

Chapter 2. Methods and Material

2.1. Experimental design

2.1.1. Study site and field methods description

Lake Lunz is a pre-alpine (600 m.a.s.l.), oligotrophic lake located in Lower Austria (47°51'N, 15°03'E) in the foothills of the Austrian limestone Alps. The lake is fed by a second-order pristine stream Oberer Seebach (OSB; hereafter termed the "inflow") (Battin, 1999; Bretschko, 1990) and discharge into downstream Unterer Seebach (USB; hereafter termed the "outflow"). The catchments soil is predominantly characterized as rendzina that is composed of spruce (*Picea abies*), larch (*Larix decidua*). (Heissenberger *et al.*, 2010), common ash (*Fraxinus excelsior),* sycamore (*Acer pseudoplatanus),* common beech (*Fagus sylvatica)* and goat willow (*Salix caprea)* (Fasching *et al.*, 2015). The area typically remains seasonally snow covered with long-term average annual air temperature 6.7^{o}C and precipitation 1608mm (Fasching *et al.*, 2015) that results fluctuated hydrograph with minimum inflow during winter and summer, and high during spring (Battin, 1999).

Three field observation sites were selected; a) Inflowing stream (Oberer Seebach), b) Outflowing stream (Unterer Seebach), and, c) Lake Lunz (epilimnion, metalimnion and hypolimnion) sampled above the deepest zone, where the floating sampling platform is positioned. Sampling resolution was monthly, three litres in volume [triplicates] and 07:00 am of the day. The lake remained ice-covered during winters therefore no samples were collected during Jan-Apr 2013, Jan 2014 and Jan-Mar 2015.

2.2. Laboratory and analytical methods

2.2.1. Fractionation processes and cryogenic storage

For POM, samples were filtered through muffled, pre-weighted Whatman^{TM} GF/C filters (1.2 µm, 47 mm Ø) using a Becker VT4.4 vacuum pump [max 150 mbar] in combination with a steel six-compartment manifold. After filtration the filters were freeze-dried, weighed for PM (seston) mass calculation and stored into 2.5mL cryogenic vials at (-80^{o}C) until lipids were extracted and analysed. For DOM, the filtrates were filtered through Teflon filters (0.2 µm, 47 mm Ø) using Becker VT4.4 vacuum pump in combination with CRAMI-Sistema manifold for the purpose of bacteria fractionation from the dissolved matter. For DOC concentration and DOM quality determination the filtrates were transferred into pre-combusted (450^{o}C - 4h) borosilicate vials and stored in dark at 4^{o}C until fluorescent spectroscopy.

2.2.2. Biochemical analysis of POM

Lipid analysis was carried out according to the methods described by McMeans *et al.* (2015) and Heissenberger *et al.* (2010).

2.2.2.1. Lipids extraction

Seston filters were transferred into small centrifuge vials and for extraction of total lipids chloroform and methanol were added, sealed under nitrogen gas flow and stored at -80^{o}C. For the organic layer separation and removal of polar impurities, ice-cold methanol, a mixture of chloroform-methanol (2:1; v/v), and NaCl-water (0.9%; w/v) solution were added and sonicated for 10 minutes using an Elma S15 Ultrasonic bath. After sonication the samples were vortexed for 1 min. using a Janke & Kunkel VF2 vortexer and then centrifuged for 5 minutes at 4^{o}C and 3000 rpm. All the samples were extracted three times and each time the lower layer containing lipids solvent was transferred into pre-muffled 8 mL vials. The solvent was concentrated to 1.5 mL by evaporating under N_2 flow (using a gas evaporator N-EVAP 111) and stored at -20^{o}C until further analysis.

2.2.2.2. Gravimetry

Gravimetric analysis was applied for determination of total lipids in POM. For this purpose tin cups (duplicates) were weighed and added with aliquots (each; 100 µL) and evaporated to dryness for at least two hours. After drying followed by 30 minutes acclimatization in room temperature the tin cups were reweighed. Mass readings of tins cups (before and after) were entered into a pre-programmed Microsoft Excel© spreadsheet, which calculated the total lipid concentration and also indicates the required volume for methylation and the required volume in the GC-vials for gas-chromatography analysis.

2.2.2.3. Fatty acid methylation

For preparation of fatty acid methyl esters (FAME), 1 mL of extracted lipids along with C19:0 internal standard evaporated under nitrogen flow until complete dryness. 1 mL of toluene (C_7H_8) was added as a solvent along with 1% solution of sulfuric acid (H_2SO_4) - methanol (CH_3OH), closed under nitrogen gas flow, and treated in a water bath [Medingen WBT6] at 50^{o}C for 16 hours. After acclimatisation 2 mL of $KHCO_3$ [2% solution] and 5 mL hexane (C_6H_{14}) were added, shaken for to release produced CO_2 and then tightened under nitrogen gas flow. The samples were vortexed very briefly and then centrifuged for 2 minutes at 4^{o}C and 1500 rpm for stabilization of aqueous phases. The upper layer as FAME were transferred into long centrifuge vial and tighten under nitrogen flow. For maximum

possible extraction of fatty acid esters the methylation process were repeated. Hexane were evaporated by placing the nitrogen evaporator assembly in an OA-SYS® water bath (35^{o}C). After drying, approximately 1.5mL of ice-cold hexane was added, transferred into labelled GC vials and evaporated under nitrogen gas flow to an indicated volume as per Gravimetry table and then stored at -80^{o}C until the gas chromatography.

2.2.2.4. Gas chromatography

Fatty acid methyl esters (FAME) were analysed using a gas chromatograph (Trace GC Ultra, Detector: FID 260^{o}C, Carrier gas: He: 1 ml/min, Detector gases: H_2: 40 ml/min, N_2: 45 ml/min, air: 450 ml/min, temperature ramp: 140^{o}C (5 min) – 4^{o}C/min – 240^{o}C (20 min) = 50 min) in synchronization with a temperature-programmable injector and an auto-sampler. A Supelco^{TM} SP-2560 Capillary Column (100 m x 0.25 mm x 0.2 µm film thickness) was used for FAME separation. Excalibur 1.4^{TM} was used for calculation and also for manual resetting of the chromatograms. Fatty acid concentrations in each sample were computed using calibration curves and peaks in reference to known standard concentrations (McMeans *et al.*, 2015).

2.2.3. Optical characterization of DOM

DOC concentrations and DOM composition were determined according to the methods being described by Fasching et al. (2015), Zsolnay *et al.* (1999), Huguet *et al.* (2009) and Coble (1996).

2.2.3.1. DOC concentration and composition

A TOC analyser (GE-Sievers 900) was used for the determination of DOC concentrations by removal of inorganic carbon. DOM fluorescence composition was characterised from fluorescence excitation-emission matrices (EEMs) using a Hitachi F-7000 spectrofluorometer. EEMs were measured at an excitation wavelength range from 240 nm to 450 nm (5-nm increments) and emission wavelength range from 250 nm to 550 nm (2-nm increments). Wavelength-dependent lamp inefficiencies were corrected using built-in correction factors. Fluorescence intensities were normalised to Raman units (r.u.) by dividing the EEM values by the area under the Raman peak (Lawaetz *et al.*, 2009). Inner-filter effects were corrected from absorbance measurements as described in (Lakowicz, 2010). From each sample, the EEM of a fresh MQ measured on the same day of analysis was subtracted as a blank.

DOM fluorophores were determined by peak picking, following the spectral regions of Coble (1996). These included peak A (λexc/λem 260/380-460 nm) and C (λexc/λem 350/420-480 nm); as indicators of humic-like substances, and B (λexc/λem 275/310 nm) and T (λexc/λem 275/340 nm) as indicators of protein-like substances.

2.2.3.2. DOM fluorescent indices

Humification Index (HIX) and Biological Index (BIX) were calculated using methods being described by Zsolnay *et al.* (1999) and Huguet *et al.* (2009). HIX is a measured ratio of two spectral region H and L, from the emission spectrum being scanned at excitation of 254 nm. The two areas (H/L) were measured between emission wavelengths 300 nm and 345 nm and 435 nm and 480 nm for L and H, respectively. HIX was used as surrogate of allochthonous DOM. BIX was measured at excitation of 310 nm through a division of fluorescence intensity emitted at 380 nm, corresponding to maximum intensity of peak M, by that at 430 nm in correspondence with the maximum peak C. Increase in BIX values (λ_{em} 380/430) is an indirect measurement of M fluorophore, which is a DOM characteristic and explains autochthonous biological activity in natural water bodies (Huguet *et al.*, 2009).

2.2.3.3. Specific Ultraviolet Absorbance ($SUVA_{254}$)

$SUVA_{254}$ was measured from the ultraviolet absorbance (Shimadzu UV 17000) of water samples at 254 nm wavelength and was normalized for DOC concentrations. $SUVA_{254}$ is an average absorption of DOC molecules, which according to Weishaar *et al.* (2003) shows very strong correlation with organic carbon aromaticity and therefore used as surrogate of aromatic carbon. The following formula was used to calculate $SUVA_{254}$:

$$SUVA\ 254 = \frac{Abs\ 254 \times \ln 10}{L \times DOC\ Conc\ (\frac{mg}{L})}$$

where L corresponds to the cuvette path length (0.05 m). $SUVA_{254}$ measured in liter per milligram carbon per meter and hence the unit is in liter per milligram carbon per meter ($L.mgC^{-1}.m^{-1}$).

2.2.4. Statistical analysis

For data entry and processing Microsoft Excel© and statistical analysis such as (but not limited to) significance tests, normality, regression, correlation and analysis of variance, R version 3.2.0 and IBM SPSS Statistics version 21 were employed. All datasets were checked for normal distribution using Skewness & Kurtosis z-values and Shapiro-Wilk's normality

test. The Shapiro-Wilk's test p values were less than 0.05 for some fatty acids and DOC signals distribution. The normality tests suggested that either the datasets had to be transformed into normal distribution for parametric tests or non-parametric tests can be run for analysis of variance and significance tests. Therefore combination of parametric one-way ANOVA and non-parametric Kruskal-Wallis-test were used for testing the hypotheses, analyses of variance among the sites and degree of significance. After parametric and non-parametric analysis of variance within the variables post-hoc multiple comparison tests were run for identifying significant variability among the prevalence of qualitative and quantitative indicators in the observation sites. Linear regression and correlation analyses were used to test the effect of inflow discharge (independent variable) on the particulate and dissolved organic matter concentrations (dependent variables).

Chapter 3. Results

3.1. Particulate matter concentration

The inflow stream (OSB) demonstrated high monthly mean particulate matter concentration (Average±SD; 3.10±4.43 $mg.L^{-1}$) with high temporal variability, as of high values during June 2013 (9.47±1.17), July 2014 (18.55±3.48) and Dec 2015 (11.91±1.28 $mg.L^{-1}$) and low during Feb 2014 (0.43±0.26), Sept 2014 (0.45±0.06) and Sept 2015 (0.55±0.32 $mg.L^{-1}$). According to Battin (1999) and Bretschko (1990) the OSB transports particulate matter with high rate of fluctuation due to rainfall, snowmelt and extreme weather events in the catchment. Pearson correlation and linear regression depict a significant and positive correlation of the stream PM concentration with the discharge ($r = 0.72$, $R^2=0.52$, *Sig. F*<0.001).

Contrary to inflow the PM concentration in the outflow remained temporally less transitional (Average±SD; 1.23±1.07$mg.L^{-1}$) with slightly distinctive seasonal peaks during June 2013 (4.41±0.25) and Nov 2014 (3.95±1.03 $mg.L^{-1}$) (Fig. 1). The concentration in the outflow stream did not demonstrate a synchronous fluctuation with the rate of precipitation and snowmelt by looking into the correlation results with the OSB stream discharge (Pearson correlation, linear regression $r = 0.24$, $R^2=0.06$, *Sig. F* =0.26).

In epilimnion of the lake highest monthly mean concentration was recorded during Oct 2013 (1.03±0.07) and Oct 2014 (1.22±0.07), and low concentration was observed during May 2013 (0.21±0.34) and Sept 2014 (0.29±0.01 $mg.L^{-1}$) (Annex. Fig. 2). Thoroughly, the particulate matter concentration in the lake did not show significant seasonal variation; epilimnion (0.56±0.29), metalimnion (0.63±0.27) and hypolimnion (0.52±.32 $mg.L^{-1}$) (p = >0.05, Annex Table 3). However, the analysis of variance and significance tests (combination of both parametric and non-parametric) explain that the particulate matter concentration in inflow, outflow streams and lake layers are significantly different from each other (p<0.01). Post hoc comparison tests after analysis of variance showed that the inflow PM mean concentration was significantly higher than epilimnion, metalimnion and hypolimnion of the lake and outflow stream (Annex. Table 3).

On average the lake received 2.41 X high particulate matter than was recorded in the outflow.

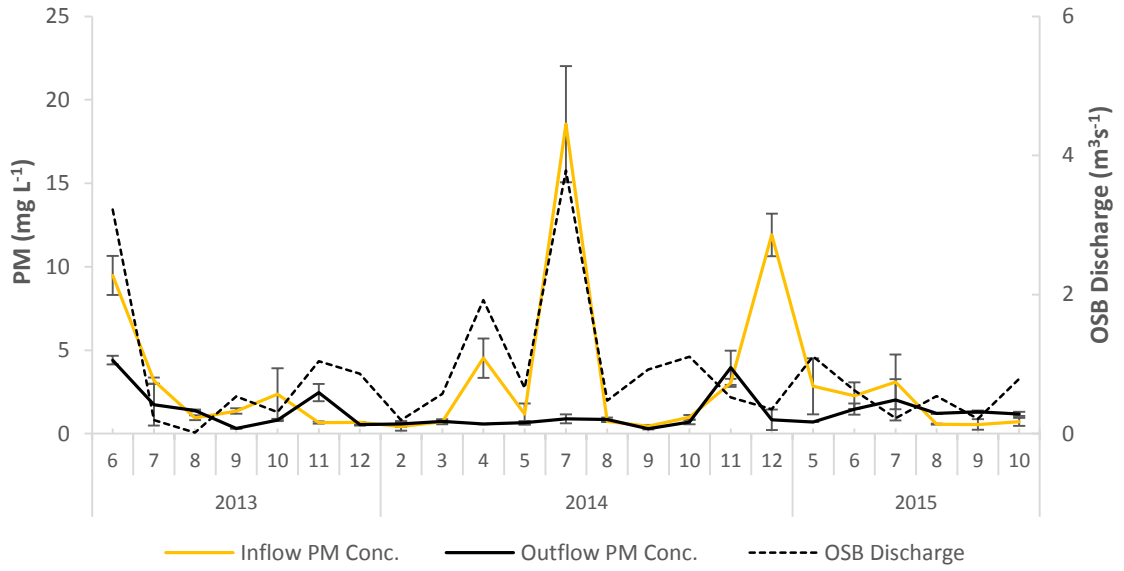

Figure 1: Concentrations of particulate matter (PM; average±St. Dev.) in lake inflow and outflow (solid lines) and average stream water inflow to Lake Lunz (dashed line) for 2013-15.

3.2. Biochemical characterization of POM

We detected 44 different fatty acids from the particulate organic matter in inflow, lake and outflow stream i.e. 14:0, 15:0, 16:0, 17:0, 18:0, 20:0, 21:0, 22:0, 23:0, 24:0, 14:1ω5, 15:1ω5, 16:1ω9, 16:1ω7, 17:1ω7, 18:1ω9, 18:1ω7, 18:1ω6, 20:1ω9, 22:1ω9, 24:1ω9, 18:1ω12, 18:2ω6, 18:3ω6, 18:3ω3, 18:4ω3, 20:2ω6, 20:3ω6, 20:3ω3, 20:4ω6, 20:4ω3, 22:2ω6, 20:5ω3, 22:3ω3, 22:4ω6, 22:5ω3, 22:6ω3, iso-15:0, anteiso-15:0, iso-16:0, iso-17:0, 16:9ω10, 18:1ω7, 18:9ω10), which according to their signatures divided into fatty acids classes viz. SAFA, MUFA, PUFA (omega-3 and -6), terrestrial fatty acids and bacterial fatty acids (BAFA).

3.2.1. Saturated fatty acids (SAFA)

The average SAFA concentrations in POM significantly varied in the inflow, lake and outflow (p<0.05, Annex. Table 3). Outflow of the lake transported POM on average containing 2.6 X higher SAFA than the inflowing POM (8.70±0.99 and 3.31±0.47 mg.g^{-1}, respectively). Apart from differences among the inflow, lake, and outflow, SAFA also varied within the lake (epilimnion, metalimnion and hypolimnion; 14.42±0.87, 13.07±1.19 and 16.08±1.77 mg.g^{-1}, respectively). The SAFA mean concentrations in the epilimnion and hypolimnion showed significant variation with the inflow and outflow (Annex. Table 3). High monthly SAFA concentrations were recorded in the outflow during Sept 2013, Sept

and Dec 2014 (14.69, 15.58 and 22.21 mg.g^{-1}) and in the lake hypolimnion during June and July 2015 (32.42 and 35.75 mg.g^{-1}; Fig. 3, Annex 1).

The mean terrestrial FA concentration (i.e., SAFA >20C) in the inflow stream was significantly lower than that in epilimnion and hypolimnion of the lake, however the mean values of all the site were significantly different (p<0.01, Fig. 4, Annex Table 3). In general, the mean monthly terrestrial FA concentrations were < 1 mg g^{-1}, except in hypolimnion during April (1.66 mg g^{-1}), July (1.41 mg g^{-1}) and Dec 2014 (1.82 mg.g^{-1}; Annex Table 1). On average, the lake received POM containing 0.29±0.05 mg g^{-1} terrestrial FA and exported 0.49 mg g^{-1} that constitute 8.8% and 5.6% of total SAFA.

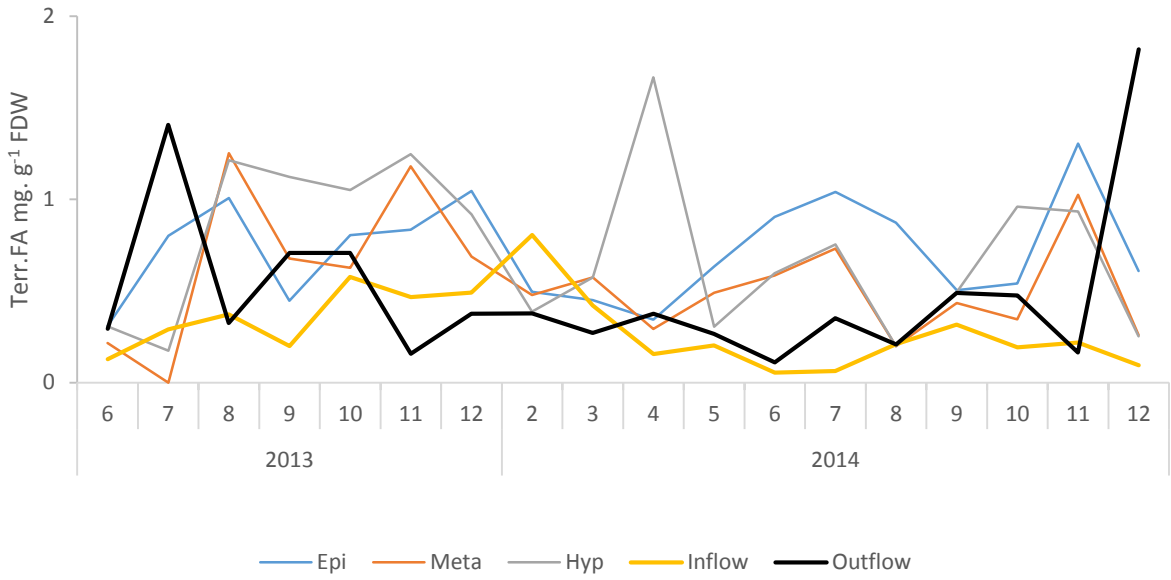

Figure 2: Mean terrestrial fatty acids concentrations of particulate organic matter at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and lake outflow (black line) for 2013-14.

3.2.2. Monounsaturated fatty acids (MUFA)

Mean MUFA concentrations in POM were significantly different among all study sites (p< 0.01). The MUFA concentration in particulate matter at outflow (4.13±2.59mg.g^{-1}) was significantly higher than of inflow stream (0.89±0.64mg.g^{-1}) (Annex Table.3). However there was no significant variation among the lake layers epilimnion (5.09±1.59mg.g^{-1}) metalimnion (4.87±2.72mg.g^{-1}) and hypolimnion (4.70±2.54 mg.g^{-1}). (Annex Table.1&3, Fig.5). Monthly mean concentration of MUFA in the metalimnion showed peaks during spring and autumn 2014, early autumn - winter 2014, and autumn 2015. The outflowing POM had 4.66 X higher MUFA contents than the inflowing.

3.2.3. Polyunsaturated fatty acids (PUFA)

PUFA concentrations in inflow, outflow streams and lake layers were significantly different from each other (p<0.05, Annex Table 3). Post hoc comparison test after analysis of variance highlights that PUFA concentration in the organic matter at inflow stream was recorded significantly lower than in the outflow stream and three layers of the lake (Fig. 6, Annex. Table 1 &3). In lake layers high PUFA concentration were observed in particulate matter during spring and summer of 2014 and 2015, however the overall monthly concentration in the lake were statistically not different (p>0.05). The lake discharged 7.4 X more PUFA in the POM than it received from the inflow.

3.2.4. Omega-3 and omega-6 PUFA

PUFA were presented as a group of omega-3 (eight types) and omega-6 (nine types) fatty acids. Here we explicitly highlight both the groups based on their potential sources of information about the organic matter diagenesis and essentiality as a resource of quality food. The average concentration of omega-3 and omega-6 PUFA in particulate matter were significantly different among all the study sites (each, p< 0.01, Annex Table 3). The mean omega-3 PUFA concentrations (inflow 0.63±0.50, epilimnion 6.97±2.46, metalimnion 6.28±3.68, hypolimnion 6.36±4.82 and outflow 5.32±3.01) were significantly higher than the omega-6 PUFA (inflow 0.31±0.24, epi 2.23±0.90, metalimnion 1.96±1.26, hypolimnion 1.77±1.35 and outflow 1.99±1.29). Post-hoc tests after analysis of variance highlighted that the average values of omega-3 (n-3) and omega-6 (n-6) in the inflowing stream were significantly lower than lake layers and outflow stream. (Annex. Fig. 7, 8 Table. 1&3). The omega-3 PUFA were ~3X higher than omega-6 PUFA (75, 76, 78, 68 and 73% of total PUFA in epilimnion, metalimnion, hypolimnion, inflow and outflow, respectively) and on average the lake discharged 7.88, 6.25 times more omega-3 and omega-6 PUFA than the inflowing.

3.2.5. Bacterial fatty acids (BAFA)

The mean BAFA concentrations were significantly different among all the sites (p<0.01). In the lake high average concentration of bacterial fatty acids was found in the seston at epilimnion, followed by the hypolimnion and metalimnion, 2.14±0.70, 2.11±1.08 and 1.93±0.93 $mg.g^{-1}$, respectively (Fig. 9, Annex. Table 1). Post hoc tests after analysis of variance showed that the BAFA concentration in the inflow was significantly lower than lake layers and outflow (Annex Table 3). Overall the seston in the outflow had 3.15 X higher BAFA concentrations than the inflow 1.34±0.73, 0.43±0.27 $mg.g^{-1}$, respectively.

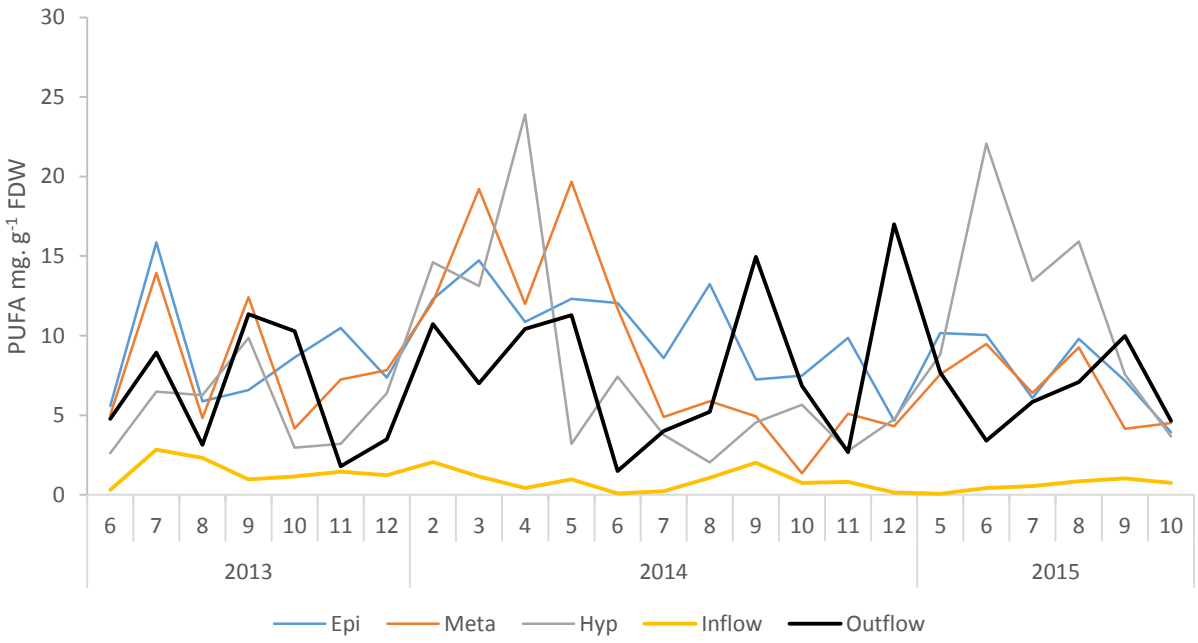

Figure 3: PUFA concentations (average) of particulate organic matter at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and lake outflow (black line) for 2013-15.

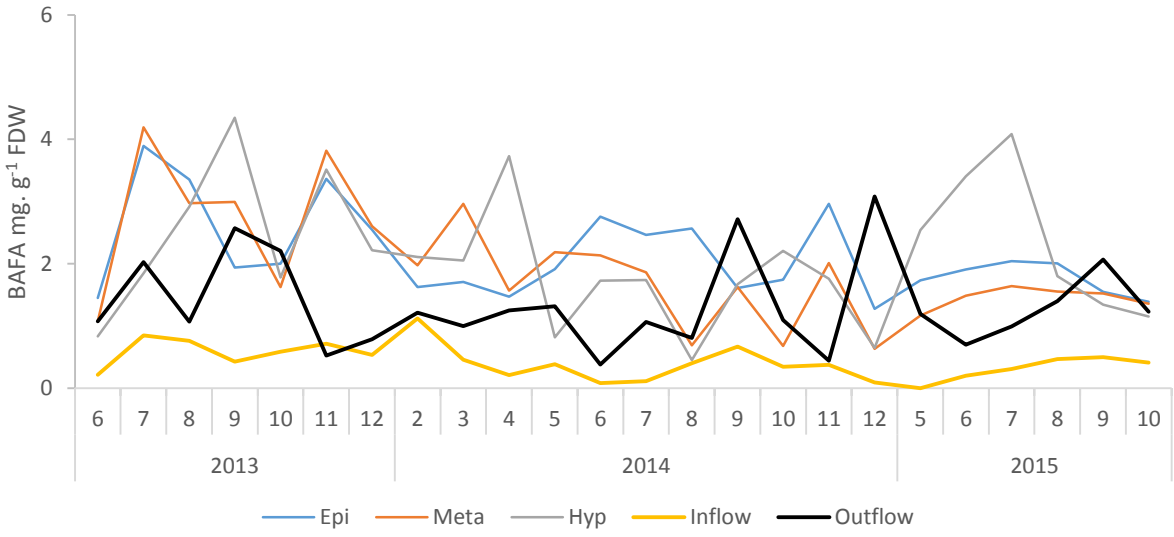

Figure 4: BAFA concentrations (average) of particulate organic matter at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and lake outflow (black line) for 2013-15.

3.3. Dissolved organic carbon (DOC) concentration

The DOC concentrations in the inflow and outflow streams remained relatively less transitional in comparison with POM concentration across the seasons. Pearson correlation and regression analysis explained that DOC concentration in inflowing stream is significantly positively correlated with OSB discharge (r = 0.4, R^2=0.16, *Sig. F* =0.04). However, unlike inflow the DOC concentration in the outflow shows insignificant correlation with the OSB discharge (r = 0.03, R^2=0.001, *Sig. F* =0.87). The monthly mean DOC concentration in outflowing stream (1.88±0.37 $mg.L^{-1}$) was relatively higher than the inflow (1.60±0.38 $mg.L^{-1}$) but statistically not significant (Annex Table 3). The vertical distribution of DOC in the lake (epilimnion 2.10±0.46, metalimnion 2.02±0.38, hypolimnion 1.85±0.36 $mg.L^{-1}$) did not vary strongly among the layers (Annex Table 3). The post-hoc test showed that the inflowing average DOC concentration was significantly lower than epilimnion and metalimnion in lake. (Fig. 10 and Annex Fig.11. Table 2).

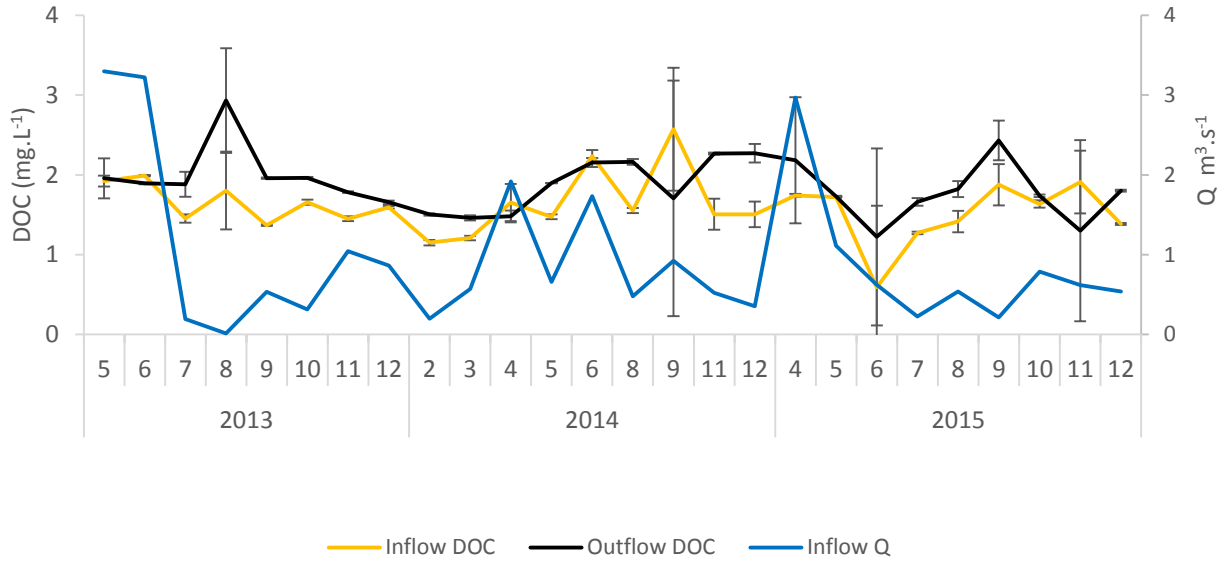

Figure 5: DOC concentration (average±SD) in Lake Lunz inflow (yellow line), outflow (black line) and inflow stream discharge (blue line) for 2013-15

3.4. DOM fluorescence characteristics

3.4.1. DOM Fluorescent signals

Intensities of four EEM fluorophores (A, C, B and T) were measured for determination of DOM fluorescence qualities. Peak A and C (humic-like substances) are used as surrogate of

allochthonous origin or recalcitrant while peak B and T (protein-like substance) as autochthonous or labile DOM.

Results of the each group of fluorophores suggest that occurrence of allochthonous DOM was higher in inflow stream, hypolimnion and metalimnion, and relatively lower in epilimnion and in the outflow. The allochthonous DOM concentration significantly varied among all the sites ($p<0.05$). The post hoc comparison test revealed that according to Peak A values there existed no significant difference between the sites while Peak C showed that epilimnion and outflow had significantly lower allochthonous DOM concentration than hypolimnion (Annex Table 3). The temporal variability was much pronounced in the outflow and epilimnion as both depicted increasing trend in allochthonous concentration during autumn and winter and descending during summer. The lake on average discharged 12.25% (peak A) and 21% (peak C) less allochthonous DOM than it received. In lake layers the hypolimnion persistently shows high values of allochthonous DOM however in epilimnion and outflow the concentration co-varied over the period of observation. (Annex. Fig. 12. Table 2).

Peak B and T (fluorophores of autochthonous DOM) values were significantly different among all the study sites (each, $p<0.01$). Comparison test between the sites showed that autochthonous DOM concentration in the inflow was significantly lower than all the three layers of the lake (Peak T) and outflow stream (Peak B) (Annex. Table 3). Both the fluorophores values demonstrated that lake on average discharged 35% more autochthonous DOM than it received (Annex Fig. 13 Table 2). Seasonal variation in the autochthonous concentration was strongly transitional in epilimnion and outflow and showed high values during July and August, respectively (Annex Fig. 13).

3.4.2. DOM Humification Index (HIX)

HIX used as a surrogate of complex and higher molecular weight recalcitrant DOM in water samples (Senesi *et al.*, 1991). HIX results of the samples depicted that inflow stream values on average remained ≤ 10 and exceeded 11 during autumn, and according to Huguet *et al.* (2009) HIX values >12 correspond to organic matter concentration from predominant terrigenous sources. Metalimnion and hypolimnion ranged 6-10 and (*ibid*) indicated this to a weak and very recent organic matter from autochthonous sources. However, epilimnion and outflow values during summer (Jun, July and Aug) were <4 and (*ibid*) indicated this as prevalence of high bacterial and algal derived DOM. In autumn (Oct, Nov and Dec), the

epilimnion and outflow values rose >4, which explains a decreasing trend of contribution from autochthonous sources. The HIX values for all the sites differed significantly (p<0.01). Post-hoc tests following analysis of variance showed that HIX values of epilimnion and outflow were significantly lower than the metalimnion, hypolimnion and inflow (Fig. 14, Annex Table 2 and 3).

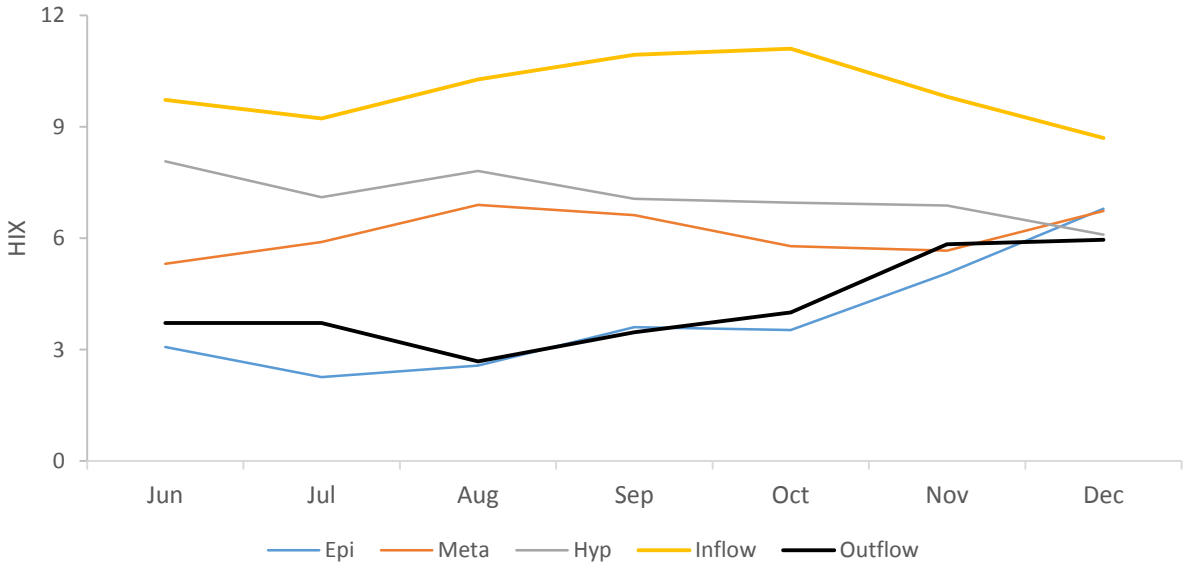

Figure 6: HIX values of DOM at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and at lake inflow (yellow line) and lake outflow (black line) for 2015.

3.4.3. DOM Biological Index (BIX)

Biological Index (BIX) is an indirect measurement of protein-like fluorophores in the dissolved pool of organic matter that surrogates autochthonous biological activity in natural water bodies or in other words labile DOM.

Huguet *et al.* (2009) ranged the BIX values from <0.6 to >1.0 and related them to corresponding magnitude of autochthonously driven DOM. The BIX values in the epilimnion were ≥0.8, which indicates very strong in situ metabolism and derived DOM. However in the metalimnion, hypolimnion and outflowing stream, the average BIX values ranged 0.7-0.8 suggesting an intermediate level while inflow stream values (0.6-0.7) very low autochthonous components of DOM. Analysis of variance revealed that the BIX values differed significantly among all the study sites (p<0.01), and sites comparison test indicated that the inflow has significantly lower autochthonous organic matter than the epilimnion and outflow streams (Fig. 15, Annex. Table 2 and 3). Seasonal trends showed that there was a

significant decrease in the autochthonous DOM at epilimnion and outflow during autumn and winter.

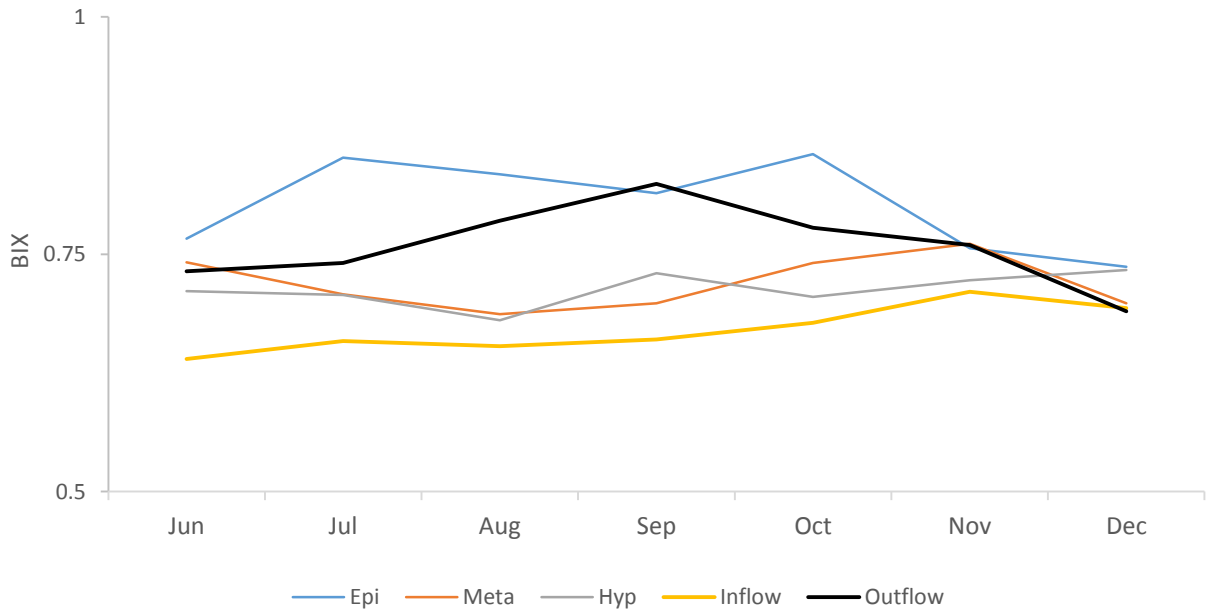

Figure 7: Biological Index (BIX) at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and at lake inflow (yellow line) and lake outflow (black line) for 2015.

3.4.4. DOM Aromaticity ($SUVA_{254}$)

Aromaticity is an indirect indicator of the terrestrial sources of DOM and determines the degree of its recalcitrance. $SUVA_{254}$ is a surrogate of aromatic character in the water and relates higher values to more aromatic carbon. The results show that epilimnion had lower $SUVA_{254}$ values (4.77±0.89 $L.mgC^{-1}.m^{-1}$) than the inflow stream (5.77±1.06, $L.mgC^{-1}.m^{-1}$) but the difference is statistically not significant ($p>0.05$).

Analysis of variance suggest that the difference in aromatic carbon concentration among the sampling sites were statistically not significant (P= 0.307). However, on average the lake discharged 8.75% less aromatic DOM than it received. The hypolimnion in the lake had relatively high $SUVA_{254}$ than metalimnion and epilimnion but were statistically not significant (Annex Fig. 15, Table 2 and 3).

Chapter 4. Discussion

4.1. POM influx/efflux

Oberer Seebach (OSB) is the main inflowing source of surface water and thus of allochthonous particulate matter into the lake. Precipitation in the catchment area cause fluctuation in the OSB discharge, which strongly alters the rate and concentration of particulate matter influx into the lake. Fasching *et al.* (2015) have found a strong correlation of organic matter concentration in the inflow with the stream discharge. According to Battin (1999) and Bretschko (1990) the OSB transports POM into the lake with high rate of seasonal fluctuation due to rainfall, snowmelt and extreme weather events in the catchment. The PM seasonal fluctuation during the three years of observations were not periodic, which may due to irregular snow melt, rainfall and duration of snow cover in the catchment. Studies have suggested that prolonged and short snow cover durations in the catchment area cause exponential changes in runoff and vegetation, and hence effect the stream discharge and POM influx (Robidoux *et al.*, 2015; Tranvik *et al.*, 2009). Contrary to inflow, the particulate matter concentration in outflow remains subtle with relative slight peaks during summer and autumn, which may be due to the lake hydraulic and particulate residence time. The epilimnion, metalimnion and hypolimnion of lake demonstrated high POM concentration during winter and low in summer, which also highlights the eminent contribution of inflowing stream. The multi-annual pattern of POM concentration in inflow, outflow and lake layers suggest that the autochthonous sources in the lake does not contribute a significant amount of POM however retains the excess and seasonally flushed inflowing terrestrial POM. Approximately 58 % of the POM entering the lake undergo biotic and abiotic degradation and sedimentation in the lake.

4.2. Biochemical characterization of POM

4.2.1. Fatty acids as biomarkers of organic matter sources

The generated results show that this lake exports about 2.6X more SAFA as POM than it receives from the inflow. This further suggests that this lake generates a substantial amount of biological driven OM in the pelagic and littoral zone and may also receive from the peripheral catchments areas. High concentration of SAFA in the outflows during autumn may be resulting from increasing inflow discharge and transportation of leaf litters from the catchment and also flushing out the recently produced and retained POM in the lake. SAFA do not help in identifying the diagenetic information of OM as it exists in all forms of life.

Long chain SAFA (>20 C) are used as a proxy of terrestrial OM (Arts *et al.*, 2009; Gladyshev *et al.*, 2013). The results showed that the lake inflow and hypolimnion had high concentrations of terrestrial FA. Terrestrial plants primarily comprise cuticular waxes with long chain SAFA (Mills *et al.*, 2001) and according to Sun *et al.* (1997) the particulate matter originating from pine and hardwood trees are mostly consisting of lignin derived FA. The reasons of the high concentration of SAFA in the hypolimnion of the lake and short chain SAFA in the outflow stream may be that the corresponding OM remained less up taken and therefore either flushed out or settled down in the sediments. Terrestrial FA form 8.8% of total SAFA in the inflow and 5.7% in outflow streams.

The higher concentration of MUFA in the lake (5 X) and in the outflow (4.6 X) than the inflow provides evidence of high biological productivity in the lake. Harwood (1996) described that high MUFA in lake and eventually in the outflow is clear evidence of algal and macrophyte biomass. Arts *et al.* (2009) mentioned the sources of fatty acid synthesis and argued that besides autotrophic MUFA sources there may be a quite substantial amount animal residuals and heterotrophic bacteria as they do have the ability to saturate short chain SAFA into their respective MUFA.

High prevalence of PUFA, especially omega-3, in the lake and outflow stream shows the same trend as MUFA, but relatively high in longitudinal gradient, which further strengthening the evidence of algal productivity in the littoral and pelagic zones of the lake. A number of studies have found high PUFA concentration in lakes as a result of phytoplankton productivity (Gladyshev *et al.* (2013); Gladyshev *et al.* (2006) and Gutseit *et al.* (2007). Algae have the ability to desaturate and elongate the low chain unsaturated FA into long chain high-saturated FA (Arts *et al.*, 2009; Krienitz *et al.*, 2006). On average the lake exported POM comprising 7.37 X more PUFA (73.3% n-3 and 27.4% n-6) than being received through the inflow stream. It suggests that the algal derived OM (autochthonously produced) is a major source of high quality carbon for the lake trophic levels and downstream aquatic and terrestrial systems. Higher concentration of n-3 PUFA in the organic matter in lake indicate availability of quality food for aquatic and terrestrial organisms (Gutseit *et al.*, 2007) and being diagnostic biomarkers suggest the abundance of fresh water algal classes, Chlorophyceae, Trebouxiophyceae and Chrysophyceae in Lake Lunz.(Gladyshev *et al.*, 2009; Taipale *et al.*, 2013).

Omega-3 PUFA are crucial biochemical constituents in diet of many organisms and is a major source of somatic and reproductive growth, and therefore play a pivotal role in

shaping the entire food web (Müller-Navarra *et al.*, 2004). Lakes with high quality of POM i.e. with high concentration of Omega-3 PUFA demonstrate high somatic growth and rate of reproduction by certain zooplanktons and crustaceans (Gutseit *et al.*, 2007; Kainz *et al.*, 2004).

Bacterial fatty acids (BAFA) were not analysed from isolated bacterial biomass, but were seen as attached to the particulate organic substrate or free individuals bigger than 1.2 µm.. High mean concentrations of BAFA were found in particulate matter at lake and outflow stream. It may be due to availability of quality food in the lake and outflow, and hence the biological productivity was higher than inflow. Sun *et al.* (1997) conducted a series of experiments and observed a positive correlation of bacterial biomass to high concentration of POM leachate from algae and macrophyte. Bacteria have the ability to de novo synthesize several important fatty acids and as being the base of food chain are transferred to the top organism in food chains through grazing and predation (Gladyshev *et al.*, 2009; Taipale *et al.*, 2013).

Overall the SAFA, MUFA, PUFA and BAFA demonstrated seasonal variation in the lake. Fatty acids contents, particularly omega-3 PUFA, in the lake exclusively depends on the algal species and nutrients availability in the lakes. Different algal species have different composition and concentration of certain FA (Krienitz *et al.*, 2006) and also nutrients concentration control the FA synthesis as Müller-Navarra *et al.* (2004) in their study found less n-3 PUFAs contents in POM with increasing concentration of total phosphorous in several lakes. Studies suggest the importance of algal derived n-3 PUFAs as being the main source of dietary derived essential FA required by aquatic animals in higher trophic levels and also terrestrial organisms such as insects, birds (Arts *et al.*, 2009) and carnivores species (Koussoroplis *et al.*, 2008).

4.3. DOC influx/efflux

Like POM the DOC concentration in the inflow was significantly correlated with OSB discharge as a direct effect of precipitation, snowmelt and dry seasons in the catchment however the outflow remained least affected due to lake buffering and residence capacity. Numerous studies have shown strong correlation between stream DOC concentration and discharge (Fasching *et al.*, 2015; Tranvik *et al.*, 2009; Wetzel, 2001). The least temporal effect of inflow discharge over the outflow DOC concentration is obvious due to the lake residence time and the same argument was given by Holdren *et al.* (2001). The mean DOC

concentration in outflowing stream (14.70% higher than inflow) suggests that the lake receives an enormous amount of DOC from base flow and also adding up the autotrophic fraction to the pool. It's hard to measure total DOC inputs into lake from the surface inflow as diffuse and autochthonous sources contribute a large fraction with high temporal and spatial variability (Hanson *et al.*, 2011). Temporal pattern showed that the inflow dissolved organic matter concentration increased during autumn, which may be due to torrential rainfall in the catchment and decreased during summer as a result of drought and short of rainfall and eventually inflow discharge. The temporal and spatial variability of DOM explained that the lake buffers the seasonal fluctuation in the DOM import and yield a regulated export to downstream system. Goodman *et al.* (2011) conducted a study on a series of subalpine oligotrophic lakes and observed the same pattern and concluded that the lakes regulate the DOC concentration and provide stability to fluvial networks in terms of water and energy regimes.

The role of Lake Lunz in sinking/processing the imported allochthonous and rate of autotrophic production of DOM may be higher than being discussed as it was assumed that the OSB is the mere influx source without being considering the ground water and peripheral runoff into the lake. The overall results suggest that the lake export 14.70% more DOC than the total OSB import. Hanson *et al.* (2011) suggest that lakes with residence time less than one year export ca. 60% of the total imported DOC. Lake Lunz residence time is 0.7 year (Malicky, 1982) and hence lead us to the speculation that the lake export ca. 96% additional DOC to the 60% of imported fraction, which may be results of lake metabolism, base flow and other diffused surface sources.

4.4. DOM fluorescence characteristics

4.4.1. Allochthonous vs autochthonous DOM

Peak A and C (humic-like substances) and HIX (degree of humification) of the DOM suggest that occurrence of allochthonous material was predominant in inflow stream and relative low in the outflow. According to Wetzel (2001) the alpine stream particularly canopied are transporting more than 90% OM of allochthonous origin. Both the indicators strongly suggest that the OSB imports a significant amount of terrigenous DOM into the lake, which results as runoff from terrestrial ecosystems and soil into the stream (Battin *et al.*, 2008; Fasching *et al.*, 2015; Tranvik *et al.*, 2009; Wetzel, 2001). Other several studies

have proved that pristine and canopied streams transport >90% of OM of allochthonous origin (Hatten *et al.*, 2012).

In lake layers the hypolimnion persistently showed high concentration of allochthonous DOM however in epilimnion and outflow the concentration co-varied over the period of observation. Huguet *et al.* (2010) also suggested that a large fraction of DOC is reactive and are being degraded in the photic zone of the lake. The sinkable OM in deep lakes goes through various degradational and transformational processes and hence the fractional that reach the hypolimnion have different properties than the one stays in suspension (Meyers & Eadie, 1993). Vähätalo *et al.* (2004) conducted long term experiments on microbial and photochemical decomposition and quantified that solar radiation accounted for 75% and 44% of the decomposition of CDOM at the surface and in the whole water column, respectively. As light attenuation in water varies spatially (vertically) and temporally hence the total and CDOM decomposition also changes accordingly. Tranvik *et al.* (2009) suggest that stable stratified lakes lead to high photodegraded epilimnetic CDOM while the hypolimnetic one as being shaded from solar radiation remain protected.

The seasonal variability was much pronounced in the outflow and epilimnion as both depicted increasing trend in allochthonous concentration during autumn-winter and descending during summer. Huguet *et al.* (2009) explained the HIX values <4 as an indication of high bacterial and algal derived DOM and the trend matches with observation of Goodman *et al.* (2011). During autumn in the epilimnion and outflow the allochthonous DOM showed an increase, which can be due to high hydraulic and terrigenous OM influx (Fasching *et al.*, 2015) and also due to decrease in the pelagic and littoral zone biological production (Wetzel, 2001).

Peak B and T (protein-like substances) demonstrated that lake produced a significant amount of algal exudates and on average discharged 35% more autochthonous DOM than the inflow. The BIX values in pretext of Huguet *et al.* (2009) affirm the high prevalence of labile DOM in epilimnion, intermediate extent in metalimnion, hypolimnion and outflowing stream, and very low concentration of labile DOM in the inflow. In situ photosynthetically produced organic matter [PDOM] is a major portion of labile source of carbon and studies suggest the high rate of respiration and assimilation by bacterial communities (Cole *et al.*, 1982; Kainz, 1997). PDOC fraction of total DOC in lakes increases with increase in algal exudation, the lysis of phytoplankton cells, and direct and indirect release by zooplankton and other aquatic

animals (Bowszys *et al.*, 2014; Wetzel, 1983). Several studies have demonstrated high bacterial biomass in causal correlation with increase in algal biomass (Vadstein *et al.*, 1989).

For further reinforcement of arguments being depicted by DOM fluorescent characteristics such as Peak A, C and HIX, the $SUVA_{254}$ was used as surrogate of aromatic fraction of DOM. $SUVA_{254}$ showed an agreement with the Peak, A, C (humic-like) and HIX and explained that inflow stream and hypolimnion had high fraction of aromatic DOM than outflow, epilimnion and metalimnion. This suggests that this lake receives the aromatic DOM from the catchment through inflow and base flow, deposit or respire a portion and export the rest.

Chapter 5. Conclusions

Assessment of the multi-seasonal and spatial qualitative and quantitative dynamics of POM lead us to the conclusion that Lake Lunz retained ~58% of low quality imported POM and regulated the export of high quality carbon enriched with 87% of autochthonously produced (omega-3 PUFA) compounds. Among other biochemical compounds in the OM, particularly omega-3 PUFA are increasingly used for assessing food quality and its dietary importance in the food chain (Masclaux *et al.*, 2014; Müller-Navarra, 1995). The lake function as a mass buffer, regulator and OM quality upgrader is also validated from the export of total DOM with an additional supplement of ~15% autochthonously produced algal exudates containing ~35% high quality carbon. Lakes, such as Lake Lunz, may in general act as organic carbon quality upgraders and regulators of seasonal fluctuation in water and organic matter mass influx.

In his paper "carbocentric limnology", Prairie (2008) discussed the progression of empirical limnology for understanding lake function and structure, and used the prediction scale of Rigler (1982) ranging from 0 (we know nothing) to infinity (know everything). Prairie speculated that by identifying phosphorous as the first limnological gradient and predictive variable in 1982, the limno-community were at 1 and progressed to 2 by the emergence of DOC as the second functional gradient. If we were to imitate this process today, I would dare to mention that by understanding the importance of omega-3 PUFA as a qualitative gradient the limnological community is proceeding beyond 2.

Gladyshev *et al.* (2009) put the argument very neatly and comprehended that despite other valuable ecosystem services one must add-on another unrecognised service i.e. **provision of PUFA to organisms in aquatic and terrestrial systems even humans**".

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Annexture

Table 1: Descriptive statistics of fatty acids in particulate organic matter (2013-15)

Descriptive Statistics	SAFA					MUFA					PUFA					BAFA				
	Epi	**Meta**	**Hyp**	**Inf**	**Outf**	**Epi**	**Meta**	**Hyp**	**Inf**	**Outf**	**Epi**	**Meta**	**Hyp**	**Inf**	**Outf**	**Epi**	**Meta**	**Hyp**	**Inf**	**Outf**
Mean	14.42	13.07	16.08	3.31	8.70	5.09	4.87	4.70	0.89	4.13	9.20	8.25	8.12	0.98	7.25	2.14	1.93	2.11	0.43	1.34
SE	0.87	1.19	1.77	0.47	0.99	0.32	0.55	0.52	0.13	0.53	0.65	0.97	1.24	0.15	0.84	0.14	0.19	0.22	0.05	0.15
SD	4.26	5.84	8.66	2.29	4.85	1.59	2.72	2.54	0.64	2.59	3.16	4.75	6.09	0.73	4.11	0.70	0.93	1.08	0.27	0.73
SV	18.17	34.07	74.93	5.22	23.51	2.53	7.37	6.43	0.41	6.69	9.99	22.58	37.11	0.53	16.92	0.49	0.86	1.16	0.07	0.53
Kurtosis	1.24	-0.59	-0.12	1.03	1.15	-1.02	4.55	-0.76	0.28	0.23	-0.54	0.70	1.19	0.62	-0.11	0.32	0.46	-0.36	0.62	0.28
Skewness	1.12	0.44	0.63	0.80	0.93	0.21	1.65	0.53	0.92	0.96	0.33	1.08	1.35	0.96	0.64	1.04	0.85	0.57	0.68	1.00
Min	8.34	4.62	3.32	0.07	1.58	2.53	1.23	0.89	0.02	1.24	3.92	1.36	2.03	0.06	1.48	1.28	0.63	0.45	0.00	0.38
Max	25.84	24.75	35.75	9.63	22.21	7.69	14.04	9.24	2.44	10.42	15.85	19.68	23.90	2.83	17.00	3.89	4.19	4.34	1.12	3.08
Count	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
Descriptive Statistics	**Terrestrial FA**					**n-3 PUFA**					**n-6 PUFA**					**n-3:n-6**				
	Epi	**Meta**	**Hyp**	**Inf**	**Outf**	**Epi**	**Meta**	**Hyp**	**Inf**	**Outf**	**Epi**	**Meta**	**Hyp**	**Inf**	**Outf**	**Epi**	**Meta**	**Hyp**	**Inf**	**Outf**
Mean	0.72	0.56	0.73	0.29	0.49	6.97	6.28	6.36	0.67	5.32	2.23	1.96	1.77	0.31	1.99	3.47	3.53	3.77	2.10	3.15
SE	0.07	0.08	0.10	0.05	0.10	0.50	0.75	0.98	0.10	0.61	0.18	0.26	0.28	0.05	0.27	0.26	0.27	0.26	0.14	0.22
SD	0.28	0.34	0.43	0.20	0.44	2.46	3.68	4.82	0.50	3.01	0.90	1.26	1.35	0.24	1.29	1.27	1.31	1.27	0.67	1.08
SV	0.08	0.11	0.19	0.04	0.20	6.04	13.52	23.22	0.25	9.03	0.81	1.58	1.83	0.06	1.68	1.62	1.70	1.61	0.46	1.17
Kurtosis	-0.67	0.07	-0.60	1.10	4.63	-0.33	1.30	0.95	0.22	0.34	-0.76	0.27	1.81	1.16	0.03	5.21	0.13	0.58	-0.20	3.80
Skewness	0.35	0.63	0.45	1.10	2.19	0.49	1.18	1.30	0.84	0.76	0.13	1.10	1.51	1.16	0.85	2.01	0.27	0.89	0.01	1.65
Min	0.31	0.00	0.17	0.05	0.11	3.20	0.56	1.62	0.04	1.10	0.69	0.59	0.41	0.00	0.38	1.92	1.02	1.92	0.71	1.85
Max	1.30	1.25	1.67	0.81	1.82	12.30	15.93	18.38	1.93	13.08	3.94	4.88	5.52	0.90	5.11	7.76	6.71	6.96	3.39	6.59
Count	18	18	18	18	18	24	24	24	24	24	24	24	24	23	23	24	24	24	23	24

SE=Standard Error, SD = Standard Deviation, SV = Sample Variance, Min = Minimum, Max = Maximum

Epi = Epilimnion, Meta = Metalimnion, Hyp = Hypolimnion, Inf = Inflow stream, Outf = Outflow Stream

Table 2: Descriptive statistics of DOM in Lake Lunz, inflow and outflow streams (DOM conc. 2013-2015), DOM quality (2015)

Descriptive Statistics	DOC Concentration mg/L					SUVA 254					HIX					BIX				
	Epi	Meta	Hyp	Inf.	Outf.	Epi	Meta	Hyp	Inf.	Outf.	Epi	Meta	Hyp	Inf.	Outf.	Epi	Meta	Hyp	Inf.	Outf.
Mean	2.10	2.02	1.85	1.60	1.88	4.73	5.49	5.68	5.77	5.27	3.84	6.13	7.14	9.97	4.20	0.80	0.72	0.71	0.67	0.76
SE	0.09	0.07	0.07	0.07	0.07	0.33	0.29	0.25	0.40	0.31	0.60	0.23	0.24	0.33	0.47	0.02	0.01	0.01	0.01	0.02
SD	0.46	0.38	0.36	0.38	0.37	0.89	0.75	0.67	1.06	0.82	1.59	0.61	0.65	0.88	1.23	0.05	0.03	0.02	0.02	0.04
SV	0.22	0.14	0.13	0.14	0.14	0.78	0.57	0.45	1.12	0.67	2.51	0.38	0.42	0.77	1.52	0.00	0.00	0.00	0.00	0.00
Kurtosis	-0.64	-0.80	1.29	2.21	1.44	1.81	1.14	-0.89	-0.62	0.26	1.08	-1.94	0.31	-1.02	-0.85	-2.08	-1.68	0.65	-0.60	0.30
Skewness	0.27	0.31	1.30	0.04	0.72	0.78	-0.39	-1.03	-0.58	-0.57	1.24	0.09	-0.05	-0.02	0.72	-0.23	0.42	-0.79	0.61	-0.11
Min	1.26	1.44	1.40	0.59	1.22	3.50	4.19	4.68	4.17	3.90	2.26	5.32	6.10	8.70	2.68	0.74	0.69	0.68	0.64	0.69
Max	2.98	2.80	2.85	2.57	2.93	6.36	6.62	6.34	7.17	6.39	6.80	6.90	8.07	11.11	5.96	0.86	0.76	0.73	0.71	0.82
Count	26	26	26	26	26	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
Descriptive Statistics	**Peak A (Humic-like)**					**Peak C (Humic-like)**					**Peak T (Protein-like)**					**Peak B (Protein-like)**				
	Epi	**Met**	**Hyp**	**Inf.**	**Outf.**	**Epi**	**Met**	**Hyp**	**Inf.**	**Outf.**	**Epi**	**Met**	**Hyp**	**Inf.**	**Outf.**	**Epi**	**Met**	**Hyp**	**Inf.**	**Outf.**
Mean	0.48	0.63	0.65	0.54	0.47	0.12	0.17	0.18	0.15	0.12	0.12	0.11	0.11	0.08	0.11	0.07	0.05	0.05	0.03	0.06
SE	0.05	0.03	0.01	0.03	0.05	0.01	0.01	0.00	0.01	0.01	0.01	0.00	0.00	0.00	0.01	0.01	0.00	0.00	0.00	0.00
SD	0.12	0.08	0.03	0.07	0.14	0.03	0.02	0.01	0.01	0.04	0.02	0.01	0.01	0.01	0.02	0.02	0.01	0.00	0.00	0.01
SV	0.02	0.01	0.00	0.01	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Kurtosis	-0.36	1.52	0.24	-1.22	0.16	-0.54	0.70	2.36	-0.02	-0.04	2.96	0.21	2.93	-1.32	-0.55	-0.72	-1.32	-2.14	-0.66	1.42
Skewness	0.58	-1.12	-1.20	0.62	1.16	0.67	-1.15	1.33	0.76	1.10	1.48	-0.08	-1.45	0.34	-0.44	0.38	0.40	-0.45	-0.45	1.34
Min	0.33	0.49	0.59	0.46	0.34	0.08	0.13	0.17	0.13	0.08	0.10	0.10	0.10	0.07	0.08	0.05	0.05	0.04	0.02	0.05
Max	0.69	0.71	0.68	0.66	0.73	0.17	0.19	0.19	0.17	0.18	0.16	0.12	0.12	0.09	0.13	0.11	0.06	0.05	0.03	0.08
Count	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7

SE=Standard Error, SD = Standard Deviation, SV = Sample Variance, Min = Minimum, Max = Maximum

Epi = Epilimnion, Meta = Metalimnion, Hyp = Hypolimnion, Inf = Inflow stream, Outf = Outflow Stream

Table 3: Analysis of variance and significance test among and between the sites

One-way ANOVA and Kruskal-Wallis p Test (***p*-value)**	**Multiple comparison after Kruskal-Wallis p Test and Tukey HSD after One-way ANOVA (Significance 0.05)**									
Among the sites	Epi-Hyp	Epi-Inf	Epi-Meta	Epi-Outf.	Hyp-Inf	Hyp-Meta	Hyp-Outf.	Infl-Meta	Inf-Outf.	Meta-Outf
PM $p<0.01$	n.s	**Sig**	n.s	Sig	**Sig**	n.s	**Sig**	n.s	n.s	n.s
SAFA $p<0.01$	n.s	**Sig**	n.s	Sig	**Sig**	n.s	**Sig**	**Sig**	**Sig**	n.s
MUFA $p<0.01$	n.s	**Sig**	n.s	n.s	**Sig**	n.s	n.s	**Sig**	**Sig**	n.s
PUFA $p<0.01$	n.s	**Sig**	n.s	n.s	**Sig**	n.s	n.s	**Sig**	**Sig**	n.s
BAFA $p<0.01$	n.s	**Sig**	n.s	**Sig**	**Sig**	n.s	n.s	**Sig**	**Sig**	n.s
Terr. FA $p<0.01$	n.s	**Sig**	n.s	n.s	**Sig**	n.s	n.s	n.s	n.s	n.s
Omega-3 PUFA $p<0.01$	n.s	**Sig**	n.s	n.s	**Sig**	n.s	n.s	**Sig**	**Sig**	n.s
Omega-6 PUFA $p<0.01$	n.s	**Sig**	n.s	n.s	**Sig**	n.s	n.s	**Sig**	**Sig**	n.s
DOC $p<0.01$	n.s	**Sig**	n.s	n.s	n.s	n.s	n.s	**Sig**	n.s	n.s
SUVA >0.05 (p =0.30)	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
HIX $p<0.01$	**Sig**	**Sig**	n.s	n.s	n.s	n.s	n.s	n.s	**Sig**	n.s
BIX p<0.01	n.s	**Sig**	n.s	n.s	n.s	n.s	n.s	n.s	**Sig**	n.s
Peak A (p=0.02)	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
Peak C $p<0.02$	Sig	n.s	n.s	n.s	n.s	n.s	**Sig**	n.s	n.s	n.s
Peak T $p<0.01$	n.s	**Sig**	n.s	n.s	**Sig**	n.s	n.s	**Sig**	n.s	n.s
Peak B $p<0.01$	**Sig**	**Sig**	n.s	n.s	n.s	n.s	n.s	**Sig**	**Sig**	n.s

n.s = Not Significant, Sig = Significant, Epi = Epilimnion, Meta = Metalimnion, Hyp = Hypolimnion, Inf = Inflow, Outf. = Outflow PM = Particulate Matter, DOC = Dissolved Organic Carbon

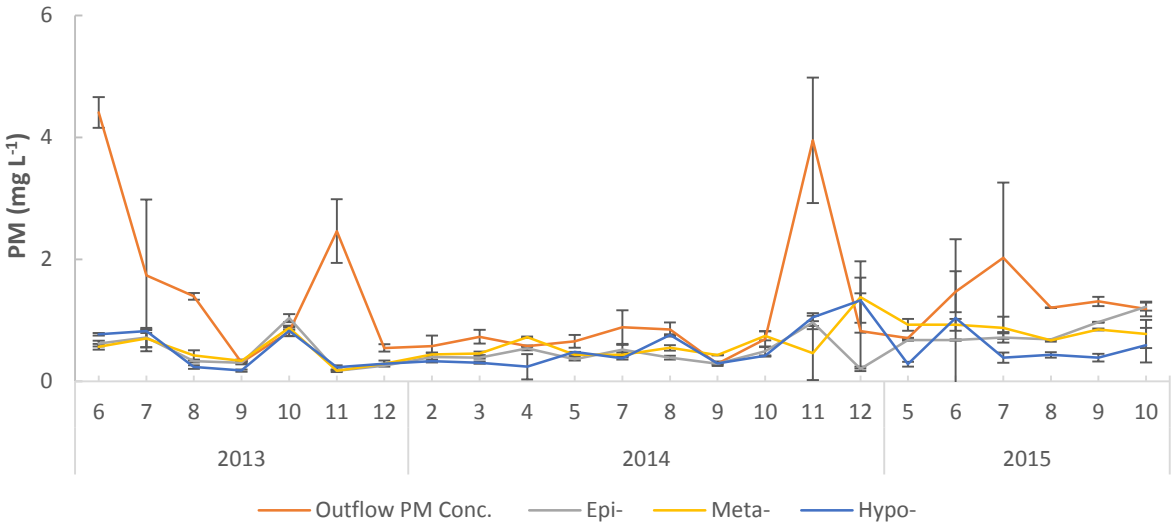

Figure 8: Concentrations of particulate matter (PM; average±SD) in lake and outflowing stream (2013-15) (Inflowing stream PM concentration is not shown here due to relative high peaks, refer **Fig. 2** for the details)

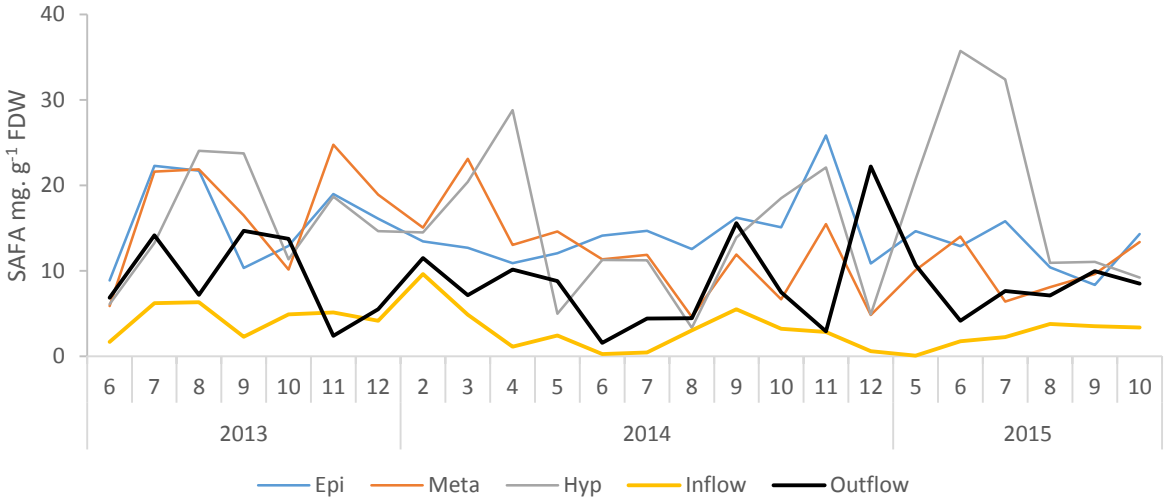

Figure 9: Mean saturated fatty acids (SAFA) concentrations of particulate matter at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and outflow (black line) for 2013- 2015

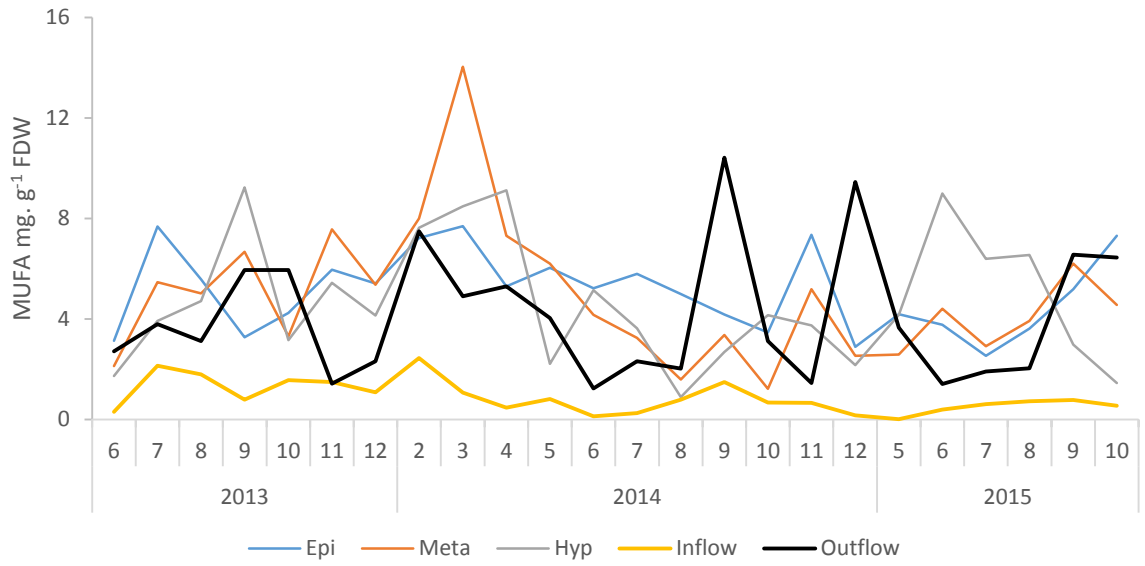

Figure 10: Mean mono-unsaturated fatty acids (MUFA) concentrations of particulate matter at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and outflow (black line) for 2013- 2015

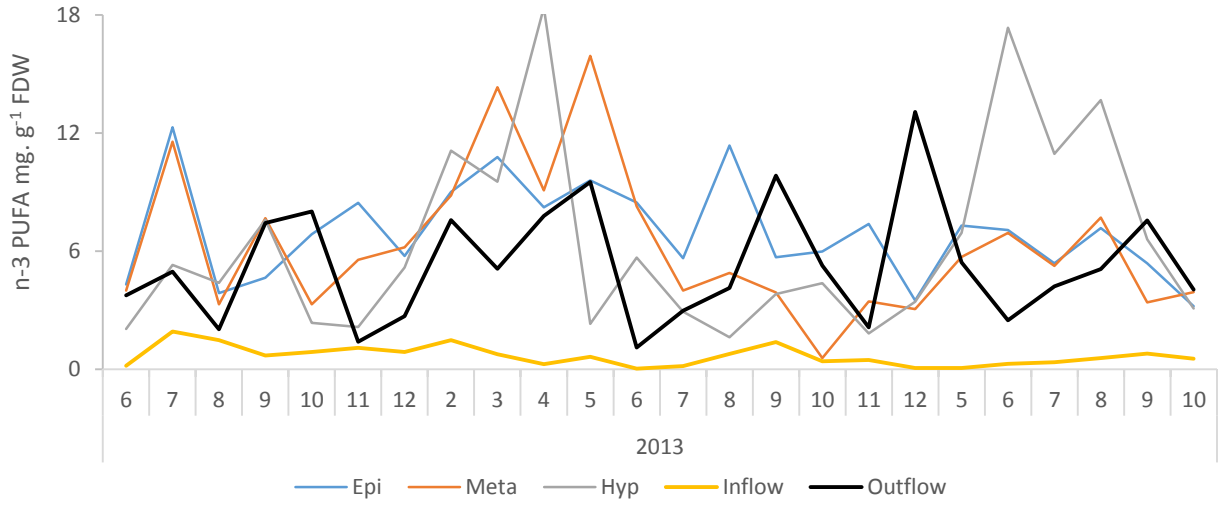

Figure 11: Omega-3 PUFA concentrations (average) of particulate matter at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and outflow (black line) for 2013- 2015

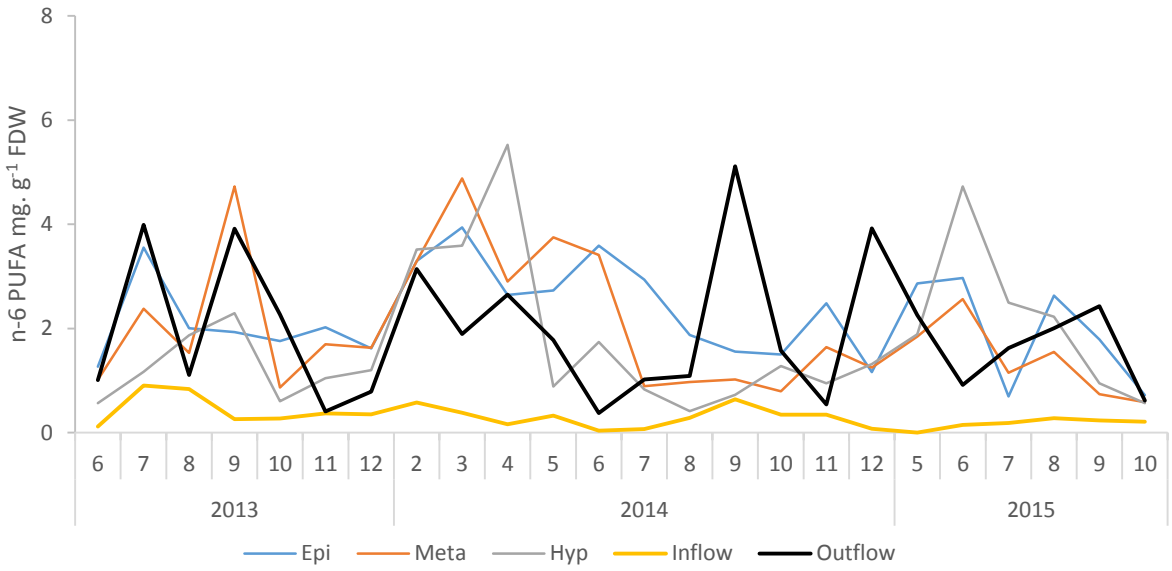

Figure 12: Omega-6 PUFA concentrations (average) of particulate matter at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and outflow (black line) for 2013- 2015

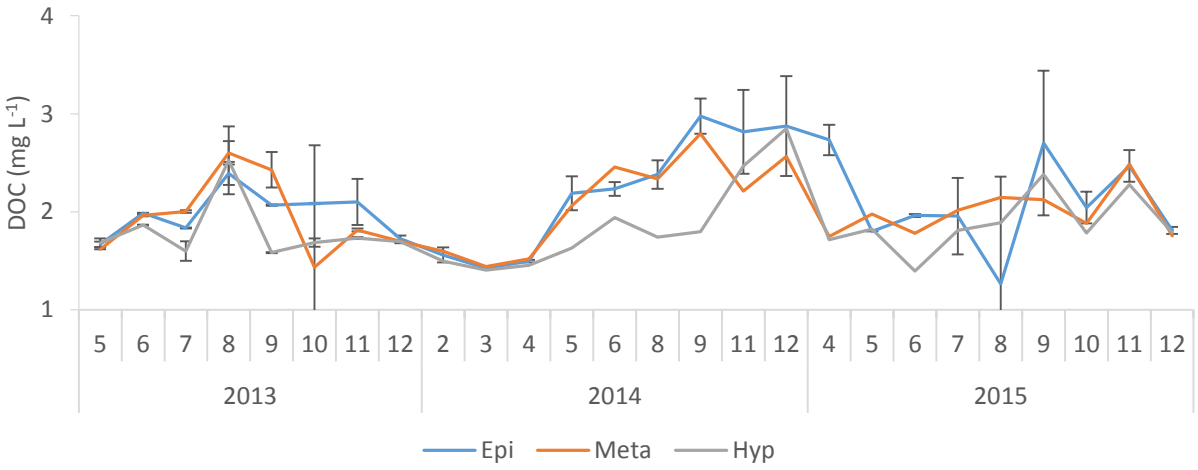

Figure 13: Concentrations of dissolved organic carbon (DOC; average±SD) at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) for 2013-15

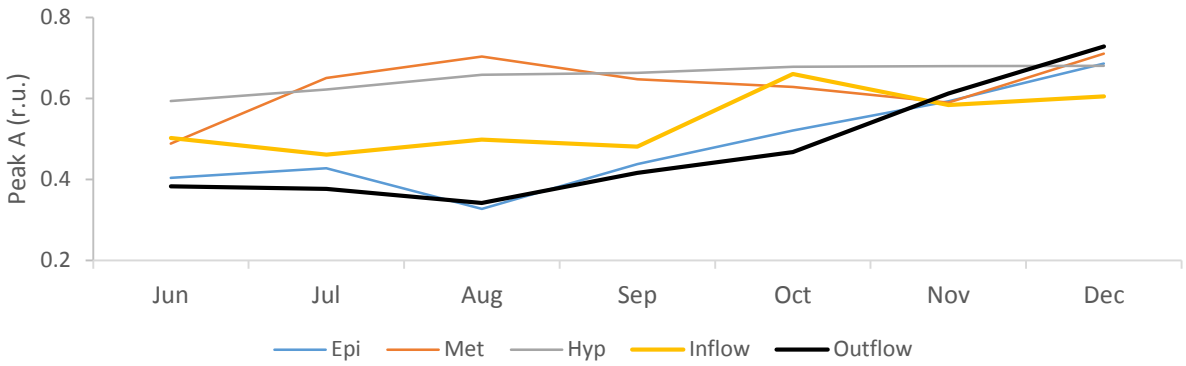

Figure 14: DOM allochthonous characteristic [Peak A] at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and lake outflow (black line) for June-Dec 2013. Note: Peak C shows the same trend for all the sites.

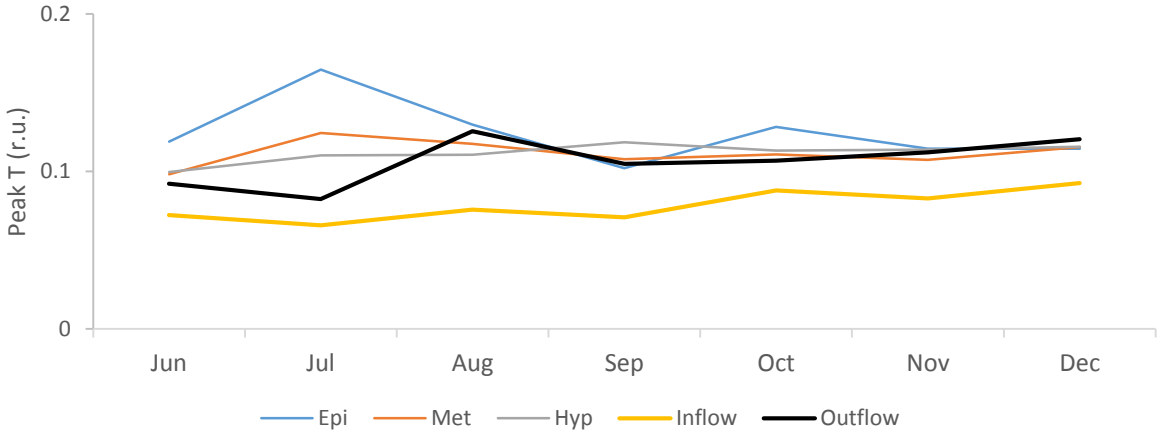

Figure 15: DOM autochthonous characteristics [Peak T] at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and lake outflow (black line) for June-Dec 2013. Note: Peak B shows the same trend for all the sites.

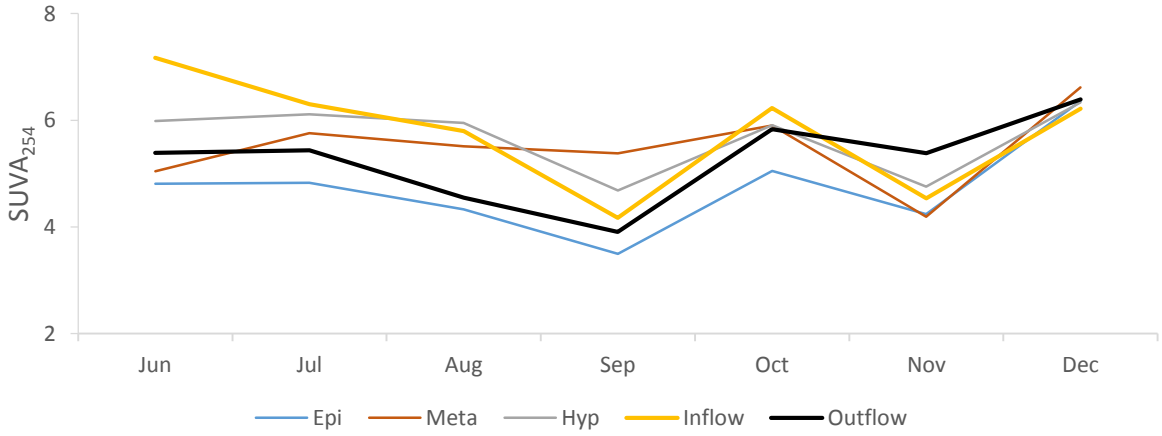

Figure 16: DOM aromaticity characterisation [$SUVA_{254}$] at 3 different depths in Lake Lunz (epi-, meta-, and hypolimnion) and in lake inflow (yellow line) and lake outflow (black line) for June-Dec 2013.