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Decreasing organic nitrogen concentrations in European water bodies - links to organic carbon trends and land cover

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Abstract

European rivers experience increasing concentrations of total organic carbon (TOC) from terrestrial sources due to factors involving changes in land use, climate and soil acidity. However, only low interest is given to the evolution of total organic nitrogen (TON) concentrations, the correlations with TOC concentrations and the influence of surrounding land cover. Investigating TON concentrations next to TOC concentrations on a wide spatiotemporal scale would also help understanding the role of TON in eutrophication episodes and its impact on water quality in general. By filtering TOC and TON annual concentration means from the Waterbase aggregated database, we gathered data from monitoring sites distributed over Europe and spread from 1990 to 2012. For each site, a concentration anomaly was calculated as the difference between a given year and a reference year (2001). This anomaly was then regressed as linear function of time to study trends in TOC, TON and TOC/TON. Through GIS and spatial analysis tools, each site was categorized into a land cover category depending on the dominant land cover falling within a 1-km buffer.
We showed that TON and TOC concentrations observed are qualified high compared to ranges observed globally for rivers and estuaries, with the highest TOC concentrations observed for "Reference" sites (>95% natural land cover) and the highest TON concentrations observed for "Urban" and "Agricultural" sites. The TOC:TON ratios observed are consequently high, regardless of the surrounding land use, with a strong positive correlation observed in ratio ranging from 0.25 to 0.40 in favor of organic C. TOC concentrations showed an increasing trend overall from 1990 to 2012, with "Urban" and "Agricultural" sites showing slightly decreasing trends and "Natural" and "Reference" sites showing steeply increasing trends. TON concentrations showed the opposite behavior with decreasing trends for all land cover categories, and particularly strong R_2 coefficient for "Urban" and "Agricultural" sites. The observed TON decreasing trends can be explained as the results of improved monitoring methodologies and policies applied by the EU and suggest optimistic consequences on the occurrence of eutrophication episodes and the improvement of water quality.

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Abbreviations

C : Carbon

CLC : Corine Land Cover

CSU : Comma-separated Values

CW : Coastal Water

DB : Database

DBP : Disinfection By-Product

DIC : Dissolved Inorganic Carbon

DIN : Dissolved Inorganic Nitrogen

DOC : Dissolved Organic Carbon

DOM : Dissolved Organic Matter

DON : Dissolved Organic Nitrogen

EEA : European Environmental Agency

EU : European Union

FPOM : Fine Particulate Organic Matter

GFF : Glass Fiber Filter

GIS : Geographic Information System

GW : Groundwater

IR : Infrared

LU : Land use

LW : Lake Water

MMU : Minimum Mapping Unit

N : Nitrogen

NDIR : Nondispersive infrared

NMR : Nuclear Magnetic Resonance

N_R : Reactive Nitrogen

NRC/LC : National Reference Centres Land Cover

ON : Organic Nitrogen

POC : Particulate Organic Carbon

POM : Particulate Organic Matter

RW : River Water

SQL : Structured Query Language

TDN : Total Dissolved Nitrogen

TN : Total Nitrogen

TO : Total Organic

TOC : Total Organic Carbon

TON : Total Organic Nitrogen

TW : Transitional Water

UV : Ultraviolet

WCO : Wet Chemical Oxidation

I. Introduction

Rising food, housing and energy demand of the increasing population creates an immense pressure on water resources around the world (Giri and Qiu, 2016), and the effects of land development on water systems is regularly assessed by studying the correlation between land use and stream water quality parameters (Reimann et al., 2009; Cunningham et al., 2010; Tran et al., 2010; Utz et al., 2011). Fast and intense land development, in the form of urban and agricultural land cover expansion, has been found to have negative impact on water quality by increasing runoff, nutrients and heavy metal loads (Pratt and Chang, 2012; Wang and Yin, 1997; Paul and Mayer, 2001; Tsegaye et al., 2006; Lee et al., 2002; Zeilhofer et al., 2010). Moreover, the dissolved organic matter (DOM) loads from urbanized rivers show characteristics strongly influenced by human development, including elevated content of chlorinated and brominated disinfection by-product (DBP) which raises health concern (Kalscheur et al., 2012; Meng et al., 2013). Thus, without monitoring, regulation and mitigation efforts, land development leads to decreasing water quality, which affects drinking water availability and highly increases its treatment cost, ecosystem stability, recreational opportunities and tourism (Mehaffey et al., 2005; Versace et al., 2008; Heathwaite, 2010; Miserendino, 2011).

Biogeochemical cycles are largely altered by human activity changes on both local and global scale. Climatic changes, via changes in temperature, hydrology, vegetation and other catchment properties, are strongly affecting the input of matter in riverine systems and, thus, recipient lakes and marine areas (Hessen et al., 2009; Huang et al. 2012). As a result, increasing carbon (C) and nitrogen (N) inputs to surface waters have been observed (Badr et al., 2008; Seitzinger et al., 2005; Wu et al., 2013). DOM increases have been observed in large parts of Europe (Berggren and Al-Kharusi, 2020), with anthropogenic dissolved organic nitrogen (DON) and dissolved organic carbon (DOC) sources from urbanized catchments observed to be significantly higher than natural sources in the United Kingdom (Miller, 1999). Particularly widespread increases in concentrations of organic carbon have also been found in eastern North America and northern and central Europe (Montheith et al., 2007).

As the main DOM constituent, the spatiotemporal variations of the DOC fraction are widely covered in the literature, whereas less attention has been given to the DON fraction. In fact, most nitrogen cycling studies focus on the inorganic forms of nitrogen (ammonium NH_4, nitrite NO_2 and nitrate NO_3) while ignoring the DON which is composed of a complex mixture including proteins, free and combined amino acids, amino sugars and nucleic acids (Badr, 2016; Huo et al., 2014; Seitzinger et al., 2002). Investigating the DON fraction in water along with other N species could provide better information on the effects of N loads in aquatic environment, such as their role in eutrophication (Huo et al., 2014; Seitzinger and Sanders, 1997; Sipler and Bronk, 2015). Moreover, recent work and improved quantification methodologies have shown that the DON fraction accounts for a large part of the total organic nitrogen (TON) fraction in many water systems (Badr et al., 2008; Seitzinger and Sanders, 1997; Sipler and Bronk, 2015). Investigating the TON fraction alongside the total organic carbon (TOC) fraction could highlight correlations between the two compounds' concentration trends and provide information on the spatiotemporal evolution of the C:N ratio (calculated [TOC]/[TON]). The C:N ratio is an important quality index that is strongly positively correlated with the aromaticity and specific light absorption (color) of organic matter produced from allochthonous processes (Yates et al., 2019). On the other hand, a low C:N ratio indicates autochthonous or anthropogenic organic matter sources with high protein contents and high reactivity (Fellman et al., 2008; Kroer, 1993). Studying the spatiotemporal variation of the ratio along with TOC and TON concentration trends could bring information on the source of DOM in surface waters and the influence of surrounding land use.

Most studies on water quality monitoring and correlations with land use are based on short term records and projected on local or basin scale (Liegel et al., 1991; Mueller et al., 2014; Jordan et al., 1997). The availability of extended data is generally sparse, making it difficult to study correlations on a wide spatiotemporal scale. It can also be pointed out that no previous large-scale study has analyzed TOC and TON trends simultaneously and their response to spatial variations in land cover. In 2019, the European Union and European Environment Agency published an open and extended data collection of more than 30 million entries of diverse water quality compound records sampled from monitoring sites distributed over Europe. To this date, the database remains poorly exploited by the scientific community and stands as an

opportunity to study surface water organic compounds on a wide spatiotemporal scale. Additionally, the Corine Land Cover (CLC) datasets are land cover data collections also produced and openly distributed by the EU, that can be used for geospatial analysis with the Waterbase database published by the EU/EEA and following common site identifiers and methodologies.

European rivers have shown decreasing inorganic N concentrations as a result to reduced use of excessive fertilizers in agriculture and better wastewater treatment (EEA, 2015, 2018). However, the organic matter concentrations continue to rise in large parts of Europe, affecting waters with eutrophication through water browning. While the importance of terrestrial DOC exports in this process is acknowledged (Hruska et al., 2009; Monteith et al., 2007; Skjelkvale et al., 2005), little is known about the influence of organic nitrogen. This project aims to investigate the fluctuations of organic N and C concentrations in European surface waters by addressing: 1) the temporal concentration trends of TOC and TON, 2) the correlation between TOC and TON concentration trends, 3) the spatial distribution of each compounds' trends, 4) the correlation between rate of concentration change and surrounding land use, 5) the spatiotemporal evolution of C:N ratio. As carbon and nitrogen are two main components of DOM, which in known to have increased widely across Europe, the DOC and DON are expected to both show increasing trends over the past few decades. The C:N ratio trend is expected to show a slow increase in time, with TOC increasing at a higher rate than TON, because of the exacerbated inputs from allochthonous sources that have relatively low nitrogen contents. However, the impact of surrounding land cover on these trends is difficult to predict based on literature, and will therefore be analyzed on an explorative basis. This project presents some of the first large-scale analyses of organic nitrogen trends across Europe.

II. Literature overview

II.1 Water quality and chemical compounds

Nutrients and chemical compounds measured to assess water quality can be classified into two categories: inorganic and organic. Organic matter can, in turn, be divided into two fractions: a dissolved and a particulate fraction. These fractions are referred to as dissolved organic matter (DOM) or particulate organic matter (POM).

II.1.1 Dissolved Organic Matter

The DOM, also referred to as natural organic matter, is largely composed of humic matter (humus) and operationally defined as any organic matter passing through a filter, as opposed to POM retained on the filter. Glass fiber filters with a nominal pore size of 0.7 µm used to be standard, but smaller-sized filter pores (0.2-0.45 µm) are often used nowadays as they remove more of the bacteria, which can degrade a DOM sample (Hartnett, 2017).

DOM is a heterogeneous class of water-soluble compounds that contain reduced (organic) carbon from a variety of biological and geological sources with a wide range of chemical reactivity (Hartnett, 2017). It originates within aquatic ecosystems, arises from groundwater sources and is imported from the surrounding terrestrial landscape (Findlay and Parr, 2017). DOM is generated from the partial decomposition of living organisms including plants, animals and soil microorganisms. Autochthonous biological production also leads to the release DOM within water systems. Processes that results in autochthonous DOM release may include phytoplankton exudation, sloppy zooplankton feeding on phytoplankton, zooplankton fecal pellet decay, bacterial degradation of algal-derived matter, viral induced cell lysis and solubilization of autochthonous detritus particles (Carlson and Hansell, 2015; Keller and Hood, 2011; Sipler and Bronk, 2014). The main sinks of DOM include bacterial uptake and mineralization, photochemical oxidation, and sorption to suspended particles (Carlson and Hansell 2015; Keller and Hood 2013).

In general, DOM includes a small proportion of low-molecular weight compounds (e.g., carbohydrates or amino acids) and a large proportion of complex and high-molecular weight compounds often referred to as humic substances. Humic substances show medium to high molecular weight and are a complex mixture of aromatic and

aliphatic hydrocarbon structures with attached amide, carboxyl, ketone and other functional groups (Leenher and Croué, 2003).

Past analysis for DOM characterization has been limited to bulk elemental analysis (DOC, DON), broad structural features from bulk elemental ratios (e.g. carbon, hydrogen or oxygen) or spectral (infrared [IR], nuclear magnetic resonance [NMR] spectroscopy or fluorescence) signatures. Ultrafiltration techniques have been used to characterize DOM by molecular weight fraction (Benner et al., 1997; Hopkinson et al., 1998).

The quantification of DOM is important as the labile forms of C and N are the primary energy sources for bacteria and other microorganisms in aquatic environments (Volk et al., 1997). Bacteria in aquatic ecosystems are the main consumers of DON and DOC, but they can also be important producers (Sipler and Bronk, 2015). They facilitate the transformation of DOM into particulate organic biomass and re-mineralize it to its inorganic forms (Kerner and Spitzy, 2001). The organic matter can be stored and decay for varying length of time, making DOM present in natural waters ranging in age from recent to thousands of years (Raymond and Bauer, 2001). The decomposition of DOM decreases in time, with the most labile compounds being metabolized at higher rates first, and less labile compounds at lower rates (Carlson and Hansell, 2015).

There is an important variability in the bioavailability and ecosystem effects of DOM (Seitzinger et al., 2005). The concentration of DOM in surface waters varies in time and space (Carlson et al., 1998; Stepanauskas et al., 2002; del Georgio and Davis, 2003; Worral et al., 2003; Evans et al., 2004; Mbaye et al., 2016; Badr, 2016; Harris et al., 2018). The response of ecosystems (e.g. coastal plankton communities) to DOM inputs also depends on the source land use (e.g. forests or urban or agricultural runoff) (Herlihy and Stoddard, 1998; Seitzinger et al., 2002; Pellerin et al., 2004; Meneses et al., 2015), the population density (Chen et al., 2016), the topography (Pratt and Chang, 2012) or the presence of animal feedlots and storage lagoons (Sun et al., 2017) for example. Rivers enrich coastal seawater with many dissolved compounds (Huang et al., 2013), and contemporary riverine inputs of DOM now amount to approximately 0.25 Gt C per year (Ribas-Ribas et al., 2011).

DOM affects aquatic ecosystems by having an influence on the acidity (Eshleman and Hemond, 1985), on trace metal transport (Lawlor and Tipping, 2003), light absorbance and photochemistry (Schindler, 1971 and Zafariou et al., 1984), energy supply (Wetzel, 1992), nutrient supply (Stewart and Wetzel, 1981), water treatment processes (Alarconherrera et al., 1994). By transferring from terrestrial to aquatic and ultimately marine ecosystems, DOM is an important component of the global carbon cycle (Hope et al., 1994).

The dissolved organic matter (DOM) is often the predominant form of organic C and N in aquatic ecosystems, while phosphorus P remains largely particulate (Findlay and Parr, 2017). For this reason, DOM is often indicative of the total organic (TO) fraction.

II.1.2 Particulate Organic Matter

The particulate organic matter (POM) or fine particulate organic matter (FPOM) is defined as any organic particles in the size range of > 0.45 to <1000 µm that are either suspended in the water column or deposited within lotic habitats (Hutchens et al., 2017). Suspended fine particulate material, also referred to as seston, includes all living (e.g., bacteria, algae, protozoans, invertebrates, etc.) and nonliving (amorphous organic matter, detritus, as well as suspended inorganic sediment) substances. It can originate from many sources, including the breakdown of larger particles by physical forces, animal consumption, microbial processes, flocculation of dissolved substances and terrestrial inputs (Wotton, 1984; Wotton, 1990). Transported loads vary greatly among lotic systems, from micrograms in some small streams to metric tons in larger streams and rivers. Seston is important to many stream ecosystem processes and represents a major pathway of organic matter transport, deposition, and export. FPOM is thus an important consideration in ecosystem organic matter budget (Fisher and Likens, 1973; Cummins et al., 1983; Golladay, 1997; Webster and Meyer, 1997; Tank et al., 2010). Instantaneous seston concentrations (e.g., mass per volume of water; mg/L) can be measured by filtering known volumes of water through pre-ashed and pre-weighted glass fiber filters (GFFs) (Hutchens et al., 2017).

II.2 Carbon

As part of the global C cycle, inland waters receive roughly 5.1 Pg of terrestrial carbon per year, which equals to approximately 70% of the global annual terrestrial net

ecosystem production (Drake et al., 2017). The riverine C flux is mainly land-derived and partly represented by dissolved organic carbon (Hedges, 1992).

II.2.1 Dissolved organic carbon

DOC is an operational measurement of the carbon content in DOM and made of a heterogeneous mix of yellow to brown or even black organic carbon compounds found in natural water in varying concentrations. The source of DOC may be either in-lake processes (autochthonous, macrophytes and phytoplankton) or from the surrounding watershed (allochthonous). DOC concentrations can range from <1 mg l^{-1} in the most transparent lakes to 50 mg l^{-1} or more (Williamson et al., 1999).

DOC can be measured following different techniques. The sample is first filtered through a GFF, silver membrane filter or a nitrocellulose/polypro filter with pore size ranging between 0.2 and 0.7 µm. The filtered-out fraction forms the particulate organic carbon (POC) fraction. Two different methods exist to purge the dissolved inorganic carbon (DIC) fraction and measure the DOC fraction from the resulting sample. The high temperature combustion method involves the conversion of inorganic carbon to dissolved CO_2. The remaining DOC is then oxidized at a high temperature, to form CO_2 which can be detected by nondispersive infrared (NDIR) sensor. The second method, or UV/Persulfate oxidation method, applies acid on the sample to lower its pH to 2.0. This process converts inorganic carbon to CO_2 which is then purged from the sample. A persulfate reagent is added to the sample to oxidize the remaining carbon into CO_2 by UV radiation. Similarly, the CO_2 can be detected by NDIR sensor (B. Schumaher, epa.gov). DOC is often referred to as synonymous with TOC, e.g. as it is represents as much as 90% of TOC across widely distributed European rivers (Berggren and al-Kharusi, 2020).

II.2.2 Dissolved organic carbon changes

The evolution of DOC concentrations are broadly documented and understood, and widespread increases in surface waters are observed in North America and in northern and central Europe (Monteith et al., 2007), including UK, the Czech Republic, Finland, Norway, Canada and USA (Worrall et al. 2004a, 2007; Evans et al. 2005, 2006; Monteith et al. 2007; Hongve et al. 2004; Skjelkvåle et al. 2001, 2005; Driscoll et al. 2003; Stoddard et al. 2003). There are different causes to explain increases of DOC,

such as simultaneous changes in atmospheric deposition of sulphur and sea salt (Monteith et al. 2007), temperature increases accentuated by land-use factors (Worrall et al. 2003), warmer climate (Tranvik et al. 2009), recovery from acidification, temperature change, hydrological change, land-use change, in-lake and in-stream removal, nitrogen enrichment, atmospheric CO_2 enrichment (Evans et al., 2005).

Increasing DOC concentrations in aquatic ecosystems influences freshwater biota, coastal marine ecosystems, upland carbon balances, drinking water quality (Evans et al., 2005) and potential for the formation of disinfection byproducts (Worrall et al., 2003).

II.3 Nitrogen

Nitrogen is one of the most important nutrients and accounts for 78% of the Earth's atmosphere as elemental N_2 gas. Elemental N_2 gas is inert, does not impact environmental quality and is not directly available for plant uptake and metabolism. Reactive nitrogen (N_r) is a term used for a variety of nitrogen compounds that are biologically or photochemically reactive in a system. The main inputs of N_r are termed as NH_x (ammonia [NH_3] and ammonium [NH_4^+]), NO_x (nitrite oxide [NO] + nitrogen dioxide [NO_2]), nitrate (NO_3^-) and nitrite (NO_2^-) (Follett and Hatfield 2001). Nitrogen is a crucial element part of enzymes structure, proteins and nucleic acids (Galloway and Cowling 2002). The inorganic compounds nitrate NO_3^-, nitrite NO_2^- and ammonium NH_4^+ can be used directly by organisms and are 'fully' bio reactive. Organic nitrogen (ON) needs to be broken down to small molecules by microorganisms and converted to inorganic forms before being used (Sadava et al. 2016). It is termed 'partly' bio reactive for this reason.

II.3.1 Dissolved organic nitrogen

Most studies of dissolved N concentrations and cycling in natural waters focus on the inorganic species (ammonium NH_4, nitrite NO_2, nitrate NO_3), and investigating the dissolved organic part will provide an improved assessment of total N loads to aquatic systems and their role in cultural eutrophication.

The quantification and characterization of DON are still challenging for waters with high concentrations of dissolved inorganic nitrogen relative to total dissolved nitrogen

(TDN) due to the cumulative analytical errors of independently measured nitrogen species (i.e., DON = TDN - NO_2^- - NO_3^- - NH_4^+/NH_3) and interference of DIN species to TDN quantification (Chon et al., 2013). However, recent work and improved methodologies such as wet chemical oxidation (WCO) and high temperature oxidation (HTO) (Badr et al., 2003; Bronk et al., 2000; Dafner and Szmant, 2014; Sharp et al., 2004) have shown that DON frequently forms the largest part of TDN in many lakes, rivers, estuarine and surface ocean waters (Badr et al., 2008; Seitzinger and Sanders, 1997; Sipler and Bronk, 2015). Overall, DON averages to 58-77% of the TON pool in aquatic ecosystems, excluding deep oceanic waters (Sipler and Bronk, 2015).

II.3.2 N cycle changes

Compared with N cycle research, the global C cycle receives a relatively larger research focus, mostly due to its pivotal impact on global warming. Nevertheless, the global N cycle shows changes that are even more pronounced, particularly in relative terms. The fertilizer industry and other various combustion processes have accelerated the transformation of atmospheric N_2 into more reactive reduced or oxidized forms of inorganic N, and various human activities currently fix more N_2 than natural ecosystems (Howarth et al., 1996; Boyer et al., 2002; Hessen et al., 2009; Vitousek et al., 1997; Galloway and Cowling, 2002; Galloway et al., 2008). This results in atmospheric reactive nitrogen (N_r) increases which cause the formation of smog and particulate matter which affect human health, terrestrial and marine ecosystems (Sutton et al., 2011).

Increased N deposition has a global impact and generate concerns for the ecological integrity and environmental health of terrestrial, freshwater and coastal marine ecosystems (Nixon, 1995; Driscoll et al., 2003; Wu et al., 2013). Nitrogen deposition has consequently increased in surface waters (Vitousek et al., 1997), contributing to riverine N exports from the temperate North American region that are 5-15 times higher than pre-industrial exports (Howarth et al., 1996). The present-day output of nitrogen from large rivers in North America and Europe to the north Atlantic is estimated to be a quadrupling of pre-industrial values (Boyer et al., 2006; Boyer and Howarth, 2008).

Atmospheric N deposition influences the concentrations of both dissolved DIN and DON in stream export from temperate forested watersheds (Hedin et al. 1995;

Campbell et al. 2000; Goodale et al. 2000; Perakis and Hedin 2002), and have an impact on aquatic and soil ecosystems by promoting acidification through increased NO_3 in surface waters (Stoddard, 1994; Henriksen et al., 1997; Eshleman and Hemond, 1985; Schindler et al., 1985; Schuurkes and Mosello, 1988; Johnson et al., 1991), eutrophication and anoxia in heavily affected areas, not only in North America and Europe but also elsewhere on the planet (Diaz and Rosenberg, 2008; Swaney et al., 2012). Additionally, N deposition affects the community and processes of ecosystems by changing the nature of elemental limitations for both autotrophs and heterotrophs in lakes and rivers. An increased N deposition over P would be an intensified limitation of P in surface waters (Hessen et al., 1997; Interlandi and Kilham, 1998; Bergström et al., 2005), or even large-scale shifts from N limitation over P limitation (Bergström and Jansson, 2006).

The eutrophication of estuaries and coastal seas is a well-understood and documented consequence of human alteration of the N cycle (Howarth, 1988; NRC 1993; Nixon, 1995; Nixon et al., 1996), and is associated to a loss of animal and plant diversity. Eutrophication can result in the multiplication and dominance of nuisance algae, characterized by toxic blooms of dinoflagellates (Anderson 1989, Burkholder and Glasgow, 1995, 1996) and brown tide organisms (Cosper et al., 1987).

Nitrate in drinking water can also represent a health concern. Microorganisms in the stomach may convert nitrate to nitrite when concentrations are high, resulting in the conversion of hemoglobin into methemoglobin, which is ineffective in oxygen transport in the blood. Elevated methemoglobin can kill children, in a condition known as methemoglobinemia (Maynard et al., 1976; Lee, 1970). High concentrations of organic carbon and nitrogen in mineral waters also have effects on metabolism (Popovych et al., 2018).

II.4 Organic nitrogen and organic carbon correlations

Few studies focused on both TON and TOC concentrations in surface waters, their potential correlation and the implications on ecosystems. Organic carbon concentrations are generally increasing in the northern hemisphere (Worrall et al., 2003; Monteith et al., 2007) and even though organic nitrogen has not been studied to the same extent, a correlation between TON and TOC is expected (Evans et al., 2005).

Understanding their spatiotemporal correlations is key to understand their sources, the effects of human development and land use changes, and plan for water management. For example, the study of the distribution and seasonality of DON and DOC in the Nile Damietta Branch showed high organic matter load from anthropogenic sources and recommended to consider DON and DOC for future water quality assessment (Badr., 2016). In the UK, anthropogenic DON and DOC sources from urbanized catchments in the Severn and the Tamar rivers appeared significantly higher when compared with the natural sources of DON and DOC (Miller, 1999). In Sweden, DOC and DON concentrations in Swedish rivers from 1987 to 2017 showed significant increases of the two compounds, with different trend coefficients and spatiotemporal gradients (Ahlgren, 2018).

II.5 Effects of land use on water quality assessment

A large number of studies have illustrated the effects of different land use and land cover changes on surface water quality, on a local or basin scale (Liegel et al. 1991; Mueller et al. 2014; Jordan et al. 1997; A. Baker 2003, Li et al. 2012; Pratt and Chang 2012; Meneses et al. 2015; Chen et al. 2016; Giri and Qiu 2016; Gu et al. 2019). Many of these studies have formed the basis or contributed to the design of water quality monitoring efforts (Puckett, 1995). While most research focuses on inorganic compounds and nutrients, only little research has focused on the relation between DOM concentrations in surface waters and land use changes. Pellerin et al. (2004) studied the role of wetlands and developed land use (urbanized and agricultural parcels) on DON concentrations and DON/TDN ratio from northeastern United States' rivers and streams data sets. Sun et al. (2017) studied bioavailability of DON in wastewaters from animal feedlots and storage lagoons. However, relationships between DOM and land use changes have rarely been studied over large regions, due to the paucity of large data sets, and the effort involved in quantifying land cover for large numbers of sites (Herlihy et al. 1998).

Several studies have documented the impact of urbanization and agriculture through land use changes on DIN concentrations and fluxes from temperate rivers and streams (Jordan et al. 1997; Valiela et al. 1997; Herlihy et al. 1998; Boyer et al. 2002). Additions of inorganic nitrogen in freshwater systems with enough phosphorus can cause eutrophication, independently or coupled to acidification (Schindler et al. 1985).

Additionally, a higher proportion of anthropogenically-derived DON is bioavailable to estuarine bacteria relative to forest-derived DON (Seitzinger et al. 2002). Therefore, urban and agricultural activity may not only alter the importance of DON in hydrologic N losses, but they may also have serious implications for our understanding of estuarine and coastal eutrophication.

III. Material and methods

III.1 Water quality data

The water quality data source used in this study is the *"Waterbase-Water Quality"* database, a corpus of six databases (Table 1) compiled by the European Environmental Agency (EEA).

Table 1 *EEA "Waterbase - Water Quality" databases and descriptions given by the EEA*

Databases	Description
Aggregated data	Annual mean values and other statistics of determinants on water quality, by monitoring site.
Aggregated data by water bodies	Annual mean values and other statistics of determinants on water quality, by water body.
Disaggregated data	Raw disaggregated water quality data on the observed values (e.g. concentrations) of determinants in rivers, lakes and groundwater as reported by EEA Member Countries on an annual basis.
Biology EQR data	Annually aggregated biological ecological quality ratio (EQR) data from rivers and lakes, by monitoring site.
Biology EQR classification procedure	Information on national classification system for each biological determinant and waterbody type, including the boundaries of ecological status classes (and of ecological potential classes, for artificial or heavily modified waterbodies).
Monitoring sites	List of monitoring site identifiers present in the WISE4 dataset tables.

The databases contain data on the status and quality of Europe's water bodies (Table 2), on the quantity of Europe's water resources, and on the emissions to surface waters from point and diffuse sources of pollution. Published in April 2019 and containing more than 33 million data entries for the disaggregated dataset, "Waterbase – water quality version 2018_1" may be the newest and largest water quality data compilation for European waters. The Waterbase database remains largely unexploited by the scientific community, making it a unique source in terms of data availability.

Table 2 *Definitions for the water bodies described in the Waterbase (Directive 2000/60/EC of the European Parliament)*

Water bodies	Variable	Description
River	RW	Body of inland water flowing for the most part on the surface of the land but which may flow underground for part of its course.
Lake	LW	Body of standing inland surface water.
Groundwater	GW	All water which is below the surface of the ground in the saturation zone and in direct contact with the ground or subsoil.
Transitional	TW	Bodies of surface water in the vicinity of river mouths which are partly saline in character as a result of their proximity to coastal waters but which are substantially influenced by freshwater flows.
Coastal	CW	Surface water on the landward side of a line, every point of which is at a distance of one nautical mile on the seaward side from the nearest point of the baseline from which the breadth of territorial waters is measured, extending where appropriate up to the outer limit of transitional waters.

The database "T_WISE4_AggregatedData" is the EEA database used in this study. It contains 3 211 183 records of mean concentration values for chemical compounds aggregated on annual samplings and categorized by monitoring sites, sampling years and water body categories amongst others. The data collection is the result of numerous national monitoring programs performed under several decades, with a wide selection of instruments and methodologies are involved. The CEN/ISO codes of the analytical methods used are provided in the database, and a description can be found on the EEA website (eea.europa.eu).

The main feature of the Waterbase is its wide spatiotemporal distribution. Most of the literature found on water quality analysis focuses on short time and localized datasets, whereas the Waterbase provides observations distributed across the European continent. This allows for trend analysis on wide time ranges, study of global geographic gradients, comparison between sites and validation with localized results. The Waterbase is the product of a global effort between European countries, all coordinated under policies and regulations from the EEA to ensure data quality.

III.1.1 Data preparation for TOC and TON (split datasets)

Two datasets were first exported from the original Waterbase, for TOC and TON annual concentration means respectively. The TOC subset contains 40453 entries sampled from 6249 monitoring sites, covering 5 water body types (coastal, groundwater, lake water, river water, transitional water) and spanning from 1973 to 2017. The TON subset contains 17402 entries sampled from 4252 monitoring sites, covering 3 water bodies (lake water, river water, transitional water) and spanning from 1970 to 2017. The figure 1 below shows the distribution of records per year for the original TOC (Figure 1a) and original TON (Figure 1b) datasets.

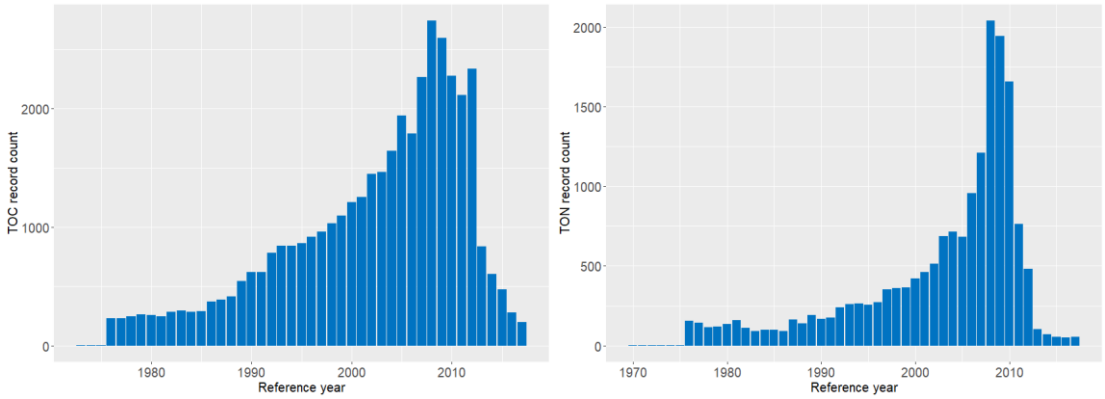

Figure 1 *Distribution samplings per given year for the a) TOC dataset (left) and b) TON dataset (right), as exported from the Waterbase without any cleaning and filtering*

Both datasets were then cleaned of all outliers (records with outstanding values), missing and non-finite values. The mean concentration records aggregated on less than 3 replicate samples per year were filtered out. The resulting TOC dataset contains 31157 entries from 5357 monitoring sites, with samplings spanning from 1976 to 2017. The TON dataset contains two times less data with 14523 entries from 3580 monitoring sites, spanning from 1970 to 2017.

For each chemical compound, two different subsets were created out of the above cleaned datasets, bringing the final number of datasets to 4 (2 for TOC and 2 for TON).

The first two subsets (1) and (2) keep all the data from monitoring sites showing at least three annual mean concentration records, regardless of the sampling time range (Figure 2a and 2b). The datasets (1) and (2) were used to calculate and chart the annually averaged concentration difference (mg l^{-1} yr^{-1}) and annually averaged rate of change

(%) for each chemical compound up to the latest known sampling years. The missing values were averaged from the under and overlaying records.

Figure 2 *Distribution of samplings per given year for the datasets a) TOC (1) and b) TON (2), after cleaning and filtering, used to graphically observed the sample distribution per year and limit the time range of study.*

The two following subsets (3) and (4) included all the data from monitoring sites having at least a measurement from a given reference year and three replicate measurements within the time range of interest. These datasets were used to calculate the concentration anomaly between a given year and the reference year for each site and regress this anomaly as a linear function of time. Because of the occurrence of records per year, the study range was set from 1990 to 2012 with 2001 as reference year for both TOC and TON variables (Figure 3a and 3b). This meant selecting the years including more than 300 records for TOC, ending up with a dataset of 15242 entries from 968 monitoring sites, spanning from 1990 to 2012 and representing 3 water bodies (Riverine, lake and transitional waters). The TON subset is focused on the same time range and reference year because of enough entries *n* (1990-2012, n > 55) and to facilitate comparison with the TOC subset. The final version includes 4609 entries from 368 monitoring sites, spanning from 1990 to 2012 and representing 3 water bodies (Riverine, lake and transitional waters).

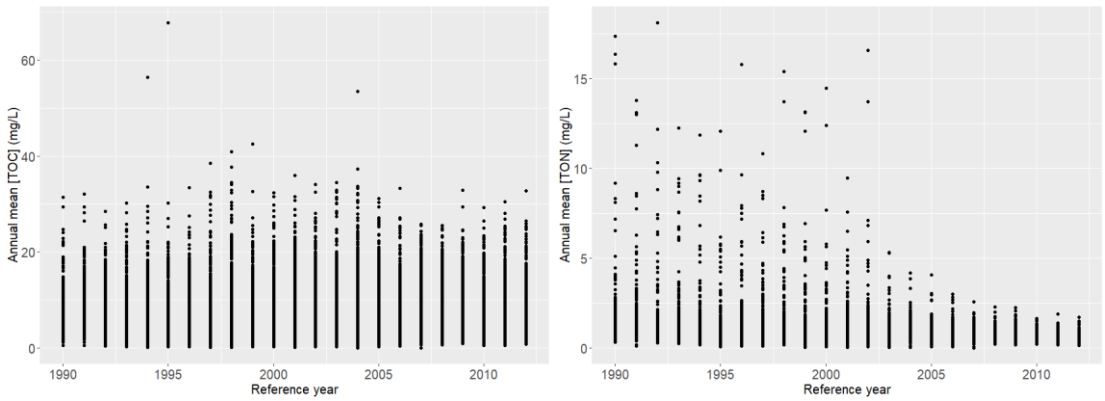

Figure 3 *Distribution of samplings per given year for the datasets a) TOC (3) and b) TON (4), after cleaning and filtering, used to graphically observed the sample distribution per year and limit the time range of study.*

In their paper from 2007, Monteith et al. selected data from the monitoring sites showing records from at least 2/3 of a given time range for their trend analysis. The same method was tested for comparison, to apprehend the effect of data manipulation on results outputted from large datasets. A TOC subset was made with only entries from monitoring sites showing at least 17 annual mean values out of the selected 26 years span (1990-2015). The final dataset contains 9247 entries, spanning from 1990 to 2015 and representing 447 monitoring sites from 7 countries. An additional TON subset was similarly created by filtering all entries from monitoring sites showing at least 15 annual mean values in this time range. The resulting dataset included 2992 entries from 170 monitoring sites only and was therefore discarded. A new dataset was exported with all monitoring sites showing 10 or more samplings between 1990 and 2012, with 4731 entries from 322 sites. The results of this study are provided as appendix.

III.1.2 Data preparation for TOC/TON (merged datasets)

Two additional datasets (5) and (6) were created from the Waterbase to study the TOC:TON ratio and correlation analysis.

The dataset (5) contains all the data from monitoring sites showing both TOC and TON annual mean concentration values (18131 entries resulting). All the outliers, non-finite and missing values were filtered out and only the entries aggregated on a minimum of 3 samples per year were kept. The resulting dataset contains 3867 pairs of TOC and TON measurements from 1170 monitoring sites, representing three water body categories (river, lake and transitional water bodies), and spanning from 1992 to 2014.

The last dataset (6) was used to study the evolution of TOC:TON ratio in time and calculate an annually averaged rate of change per site. Only the years showing more than 100 records were kept, limiting the study range from 2002 to 2012 and with 2007 as reference year. Finally, only the entries from monitoring sites showing more than 3 pairs of records [TOC]:[TON] in the study range and including a record on the reference year 2007 were kept. The final dataset contains 1573 entries from 248 monitoring sites and spanning from 2002 to 2012.

Table 3 *List of all the datasets created for this study from the Waterbase*

Dataset no.	Chemical compound	Purpose
1	TOC	Annually averaged concentration difference and rate
2	TON	Annually averaged concentration difference and rate
3	TOC	Time trend with anomaly regressed from time
4	TON	Time trend with anomaly regressed from time
5	TOC/TON	TON regressed from TOC
6	TOC/TON	Annually averaged rate of change

III.1.3 Land cover data

The Corine Land Cover (CLC) inventory was initiated in 1985 and the first dataset released in 1990. Updates have been produced in 2000, 2006, 2012 and 2018. The dataset charts land covers in Europe in 44 classes, organized into 15 subcategories and 5 categories (Artificial, Agriculture, Forest, Wetland and Water). CLC uses a Minimum Mapping Unit (MMU) of 25 hectares for areal phenomena and a minimum width of 100 meters for linear phenomena. The inventories involve 26 countries for the 1990 edition and up to 39 countries from the 2012 and 2018 editions.

The Eionet network National Reference Centres Land Cover (NRC/LC) is producing the national CLC databases, which are coordinated and integrated by the European Environmental Agency. CLC is produced by most countries by visual interpretation of high resolution satellite imagery. In a few countries semi-automatic solutions are applied, using national in-situ data, satellite image processing, GIS integration and generalization.

The CLC dataset from 2012 was downloaded from the Copernicus programme website in raster format for this project. The raster contains land cover data with a resolution of 100 meters over Europe. The 44 land cover categories have been reclassified into four,

to isolate monitoring sites into clusters and better identify relationships and correlation with chemicals' concentrations (Figure 4). The reclassification was done in Arcmap and the resulting classes are:

1. Artificial surfaces (Urban fabric; Industrial, commercial and transport units; Mine, dump and construction sites; Artificial, non-agricultural vegetated areas)
2. Agricultural areas (Arable land; Permanent crops; Pastures; Heterogeneous agricultural areas)
3. Natural areas (Forests; Scrub and/or herbaceous vegetation associations; Open spaces with little or no vegetation; Inland wetlands; Maritime wetlands)
4. Water bodies (Inland waters; Marine waters)

The fourth category "Water bodies" was disregarded as it does not tell us about land use and chemical compounds transportation.

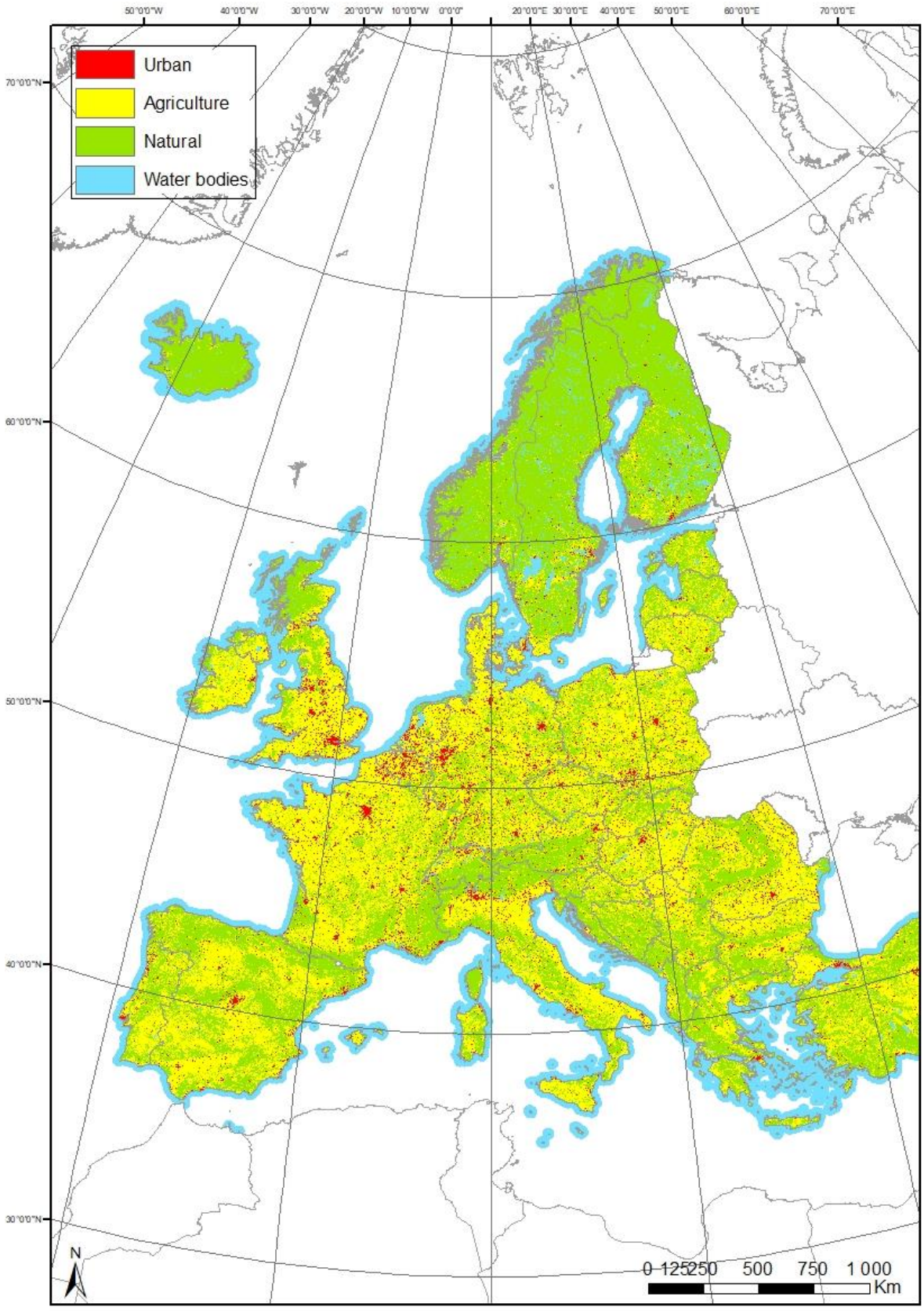

Figure 4 *Chart of CLC dataset after reclassification of the land use classes into 4 main classes*

III.2 Methods

The Waterbase aggregated database was downloaded in SQLite format and handled in DB Browser for SQLite software (v3.11.2, QT v5.11.3, SQLite v3.27.2). The first exports from the Waterbase were made through SQL script in DB Browser. The resulting datasets were exported to CSV (Comma-separated values) and all data preparation (cleaning, filtering, editing) and statistical analysis were done with R (R Development Core Team, 2013) under RStudio (v1.3.1073).

The methodology used for time trend analysis is inspired by Berggren and Al-Kharusi (2020) framework, by calculating the difference (anomaly) between a given year and a reference year for each monitoring site, then regressing the anomaly as linear function of time. It must be noted that this study focuses on a fixed time range (1990-2012) to focus on the years with a high number of records, when Berggren and Al-Kharusi considered all anomalies without any time limits. The regression lines are weighted on the mean values of each annual distributions.

The graphical representation of TOC and TON annual mean concentration exported from the Waterbase clearly showed skewed normal distributions. The log-transformed variables were therefore kept for all the latter analysis. All graphical representations for normal and log-transformed distributions are provided as appendices.

Each entry of the database was ordered by monitoring site in alphabetical order and years of sampling in increasing order. A time step was calculated between the sampling year of an entry and the lagging one, for all monitoring sites. Similarly, a chemical concentration difference and difference ratios were calculated between a given annual record and the previous one. Concentration differences and rates of change were averaged by dividing their sums with the total time lag, and results were assigned to their unique site identifiers in a new table. The dataset was joined to the "MonitoringSite_DerivedData" table with relation to the EEA unique site identifiers, to assign each site with its corresponding pair of geographic coordinates (Lat/Long, WGS84). For secrecy reasons, some of the monitoring sites' coordinates are not provided by the EEA.

The data studied is widely distributed over Europe, making it difficult to relate each monitoring site with the land use in its respective hydrological catchment. Instead, only the land use surrounding each site within a buffer of 1 km was considered. After several tests were made with different radius values (0.5 km, 2.0 km) without affecting the end results, the buffer of 1 km was kept. The Corine Land Cover 2012 was used to assign land use fractions to each site with zonal statistics tool from Arcmap. The CLC 2012 dataset was downloaded as a raster with a resolution of 100 meters.

The tool "Tabulate Area" in Arcmap (ESRI, v10.7.1) was used to assign each monitoring site with the surface of each land use category in its surrounding buffer. The resulting table was exported and formatted in R, with statistics on land cover around sampling points provided in the result section.

Each monitoring site was assigned fractions of land use categories by association with its unique site identifier. All the sites showing more than 95% natural land use were considered reference sites. The remaining were categorized following their dominant land use fraction. The distribution of log-transformed annual means was plotted by land use categories.

After data cleaning and filtering, the log-transformed annual mean concentrations of TON were regressed on TOC. Each entry was then assigned a pair of geographical coordinates and land use category fractions through zonal statistics. Annual mean log[TON] was again regressed on log[TOC] per land use categories.

A last dataset was exported to study the evolution of [TOC]/[TON] ratio in time, which includes all entries from monitoring sites with at least 3 samples. All the entries were ordered by monitoring sites and sampling years. The time step was calculated as the difference between a given sampling year and the lagging one. The ratio difference and difference rate were calculated following the same technique. Two new tables were created to assign each monitoring site with an annually averaged TOC:TON difference and rate of change.

IV. Results

IV.1 Total Organic Carbon

IV.1.1 TOC annual mean concentrations

For the dataset TOC (1) created to calculate annual differences and rates of change, the mean ± one *SD* log[TOC] was 1.74 ± 0.82 (= antilog 7.54 [7.47 – 7.60] mg/l). For the dataset TOC (3) created to calculate time trends, log[TOC] averaged 0.77 ± 0.37 (= antilog 7.82 [7.74 – 7.90] mg/l) (Table 3). The boxplot representations graphically confirm similar statistics between datasets (1) and (3) (Figure 5).

Table 4 *Statistics on the annual mean TOC concentration (mg/L) from the datasets created*

Variables	Minimum	1st Qu.	Median	Mean	3rd Qu.	Maximum
TOC (1)	0.000	3.529	6.517	7.536	10.141	90.771
TOC (3)	0.000	3.900	7.076	7.821	10.550	67.742

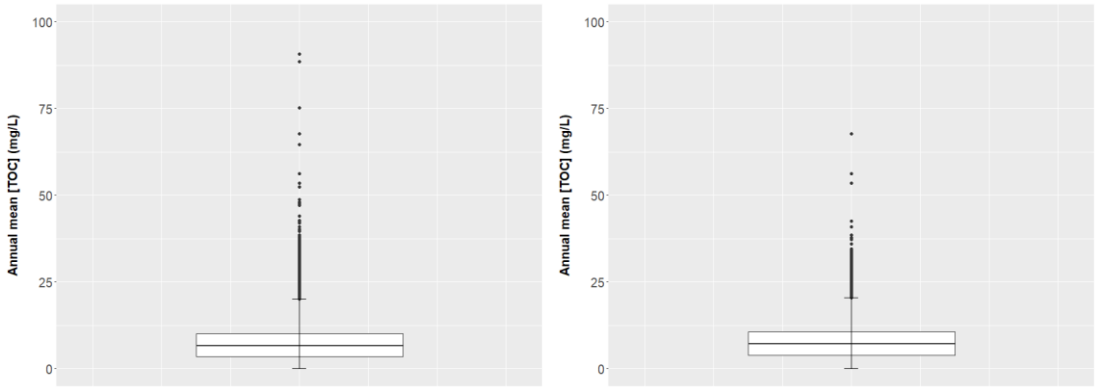

Figure 5 *Box and whisker plots summarizing the distribution of annual mean TOC records (mg/l) from a) TOC (1) (left) and b) TOC (3) (right). Boxes show the median values +/- one quartile, whiskers show min/max values within 1.5 times interquartile range below/above quartiles and point*

From a similar dataset created from the Waterbase, Berggren and Al-Kharusi (2020) observed log[TOC] averaging 0.63 ± 0.36 (= antilog 4.3 [1.9-9.9] mg/l) and a DOC fraction making up 89% of TOC. The same conversion ratio ($C_{TOC} = C_{DOC} / 0.9$) was observed from another study of ca. 7500 lakes widely distributed (Sobek et al., 2007). Following the same conversion ratio, the annual mean DOC would approximate 5.87 ± 4.88 mg/l and 6.37 ± 4.79 mg/l for TOC (1) and TOC (3) respectively. The results observed reflect high organic loads that are consistent with surrounding human activities. They depict the normal river as mesotrophic to slightly eutrophic, with the whole range from ultraoligotrophic to hypereutrophic systems represented in the dataset.

IV.1.2 Characterization of TOC monitoring sites

For all monitoring sites from the subset TOC (3) (*n* = 968), the surrounding land use averaged 17.29% Urban, 30.97% Agricultural, 41.61% Natural and 10.13% Water dominant types. Following the fraction of dominant LU, 166 sites (17%) were categorized as "Urban", 343 sites (35%) as "Agriculture", 391 (40%) as "Natural" and 62 (6.4%) as "Reference". The remaining 6 sites with a dominant "Water" LU type were discarded. The sites classified as "Reference" showed the highest results with a mean ± one SD log[TOC] averaging 0.93 ± 0.35 (= antilog 10.75 [10.35 – 11.14] mg/l). The "Natural" sites showed the second highest value with mean averaging 0.79 ± 0.36 (= antilog 8.1 [7.97 – 8.23] mg/l). The "Urban" and "Agriculture" sites showed the lowest means with 0.75 ± 0.37 (= antilog 7.47 [7.28 – 7.67] mg/l) and 0.72 ± 0.36 (= antilog 7.04 [6.89 – 7.18] mg/l) respectively (Figure 6).

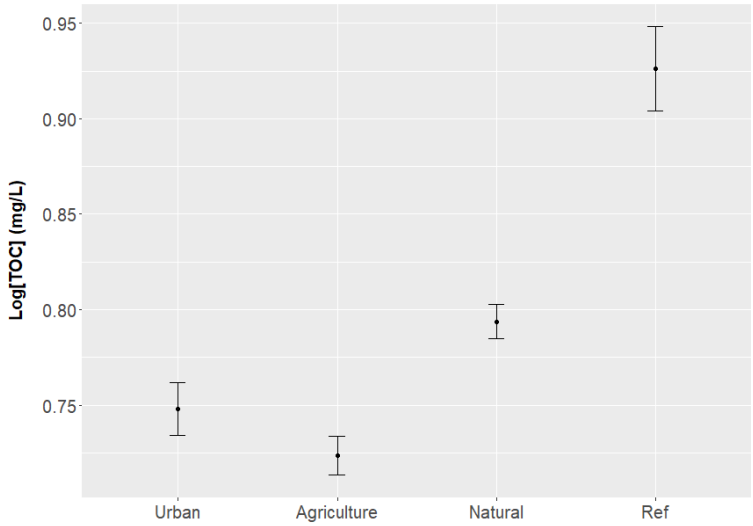

Figure 6 *Mean (points) ± 95% confidence interval of measured log-transformed TOC concentrations (mg/L) and classified per dominant land use types.*

IV.1.3 TOC trends

The log[TOC] anomaly relative to the reference year 2001 increased from 1990 to 2012 (R^2 = 0.42, p <= 0.001), with a linear trendline showing a global increase of 0.05 log units (+12% in absolute TOC) during the period (Figure 7). For sites with the dominant "Urban" land use, the log[TOC] anomaly remained stable from 1990 to 2012 (R^2 = 0.00048, p = 0.921). The dominant "Agriculture" land use type also showed a stable anomaly from 1990 to 2012 (R^2 = 0.036, p = 0.383). Monitoring sites with the dominant "Natural" type showed an anomaly increasing under the time period (R^2 = 0.8, p <=

0.001) with a linear trendline increasing by 0.10 log units (+26% in absolute TOC). The "Reference" monitoring sites followed the same previous observation with an anomaly strongly increasing under the time period (R^2 = 0.91, p <= 0.001) and a linear trendline increasing by 0.17 log units (+46% in absolute TOC). All the graphics for trend analysis categorized per land use types are provided as appendices (Figure A-5 to A-8).

Figure 7 *Anomaly relative to 2001 in log-transformed values of TOC per monitoring sites. Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

IV.1.4 TOC annual difference and rate of change

For all monitoring sites *n* from the subset TOC (1) excluding the dominant land use "Water" (*n* = 3123), the difference averaged -0.02 ± 0.69 [-0.14 – 0.17] mg/l/year, and the annual rate of change averaged 5.52 ± 25.31 [-0.97 – 6.33] %/year. The "Urban" sites averaged an annual concentration difference of -0.009 ± 0.71 [-0.154 – 0.146] mg/l/year, "Agriculture" sites averaged -0.077 ± 0.797 [-0.206 – 0.177] mg/l/year, "Natural" sites averaged 0.036 ± 0.493 [-0.060 – 0.159] mg/l/year and "Reference" sites averaged 0.144 ± 0.710 [-0.154 – 0.146] mg/l/year. These results show the same pattern as mean concentration results, with the highest and positive differences observed from "Natural" and "Reference" sites, highlighting an active and increasing input of labile organic matter from terrestrial sources. However, the spatial distribution of annual differences and rate of change did not show any pattern or geographical gradient (Figure 8).

A total of 1484 sites showed a negative annually averaged differences, 1636 showed positive differences and 3 showed no variation. Of all sites, 175 showed significant increase (>= 25%/year) and 10 showed a significant decrease (<= -25%/year). Most positive differences align with the increasing trends strongly influenced by the input of terrestrial organic matter from “Natural” dominated sites.

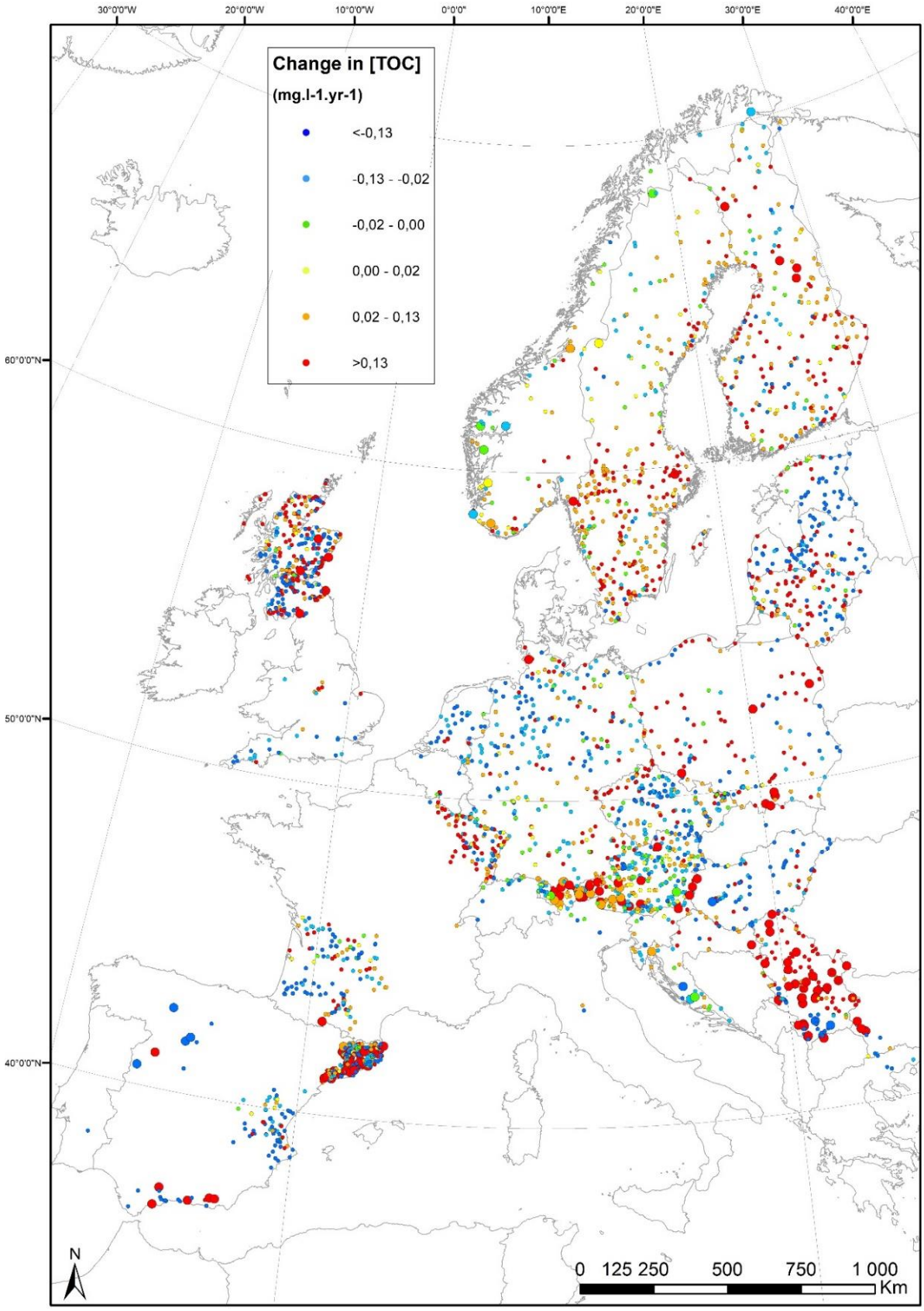

Figure 8 *Trends in total organic carbon (mg. l^{-1} .yr^{-1}). Symbol size varies with the yearly averaged concentration rate of change (+/- 25%). Data are shown for monitoring sites from the Waterbase showing mean concentration records on a minimum of three different years.*

IV.2 Total Organic Nitrogen

IV.2.1 TON annual mean concentrations

The first dataset TON (2) created to calculate annual differences and rates of change showed a mean ± one SD log[TON] was -0.11 ± 0.38 (= antilog 1.18 [1.15 – 1.20] mg/l), and the second dataset TON (4) for time trend analysis showed log[TON] averaging -0.06 ± 0.33 (= antilog 1.21 [1.16 – 1.25] mg/l) (Table 5). The boxplot representations for the datasets (2) and (4) confirm graphically similarities between the two datasets (Figure 10).

Table 5 *Statistics on the annual mean TON concentration (mg/L) from the datasets created*

Variables	Minimum	1st. Qu	Median	Mean	3rd Quartile	Maximum
TON (2)	0.0000	0.4870	0.8009	1.1765	1.2531	18.9541
TON (4)	0.0000	0.5761	0.8580	1.2063	1.2963	18.0585

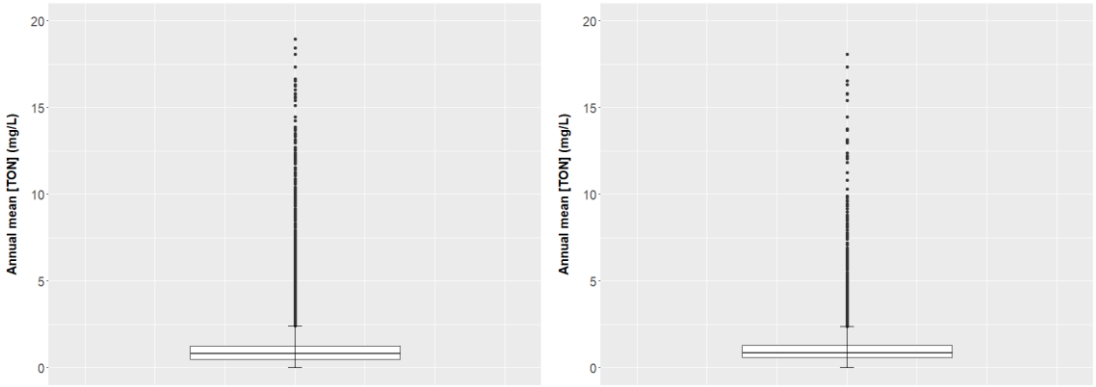

Figure 9 *Box and whisker plots summarizing the distribution of annual mean TON records (mg/l) from a) TON (2) (left) and b) TON (4) (right). Boxes show the median values +/- one quartile, whiskers show min/max values within 1.5 times interquartile range below/above quartiles and point*

IV.2.2 Characterization of TON monitoring sites

For all the sites *n* from the subset TON (4) (*n* = 368), the surrounding land use (1 km buffer) averaged 19.57% of Urban type, 51.79% Agricultural, 23.64% Natural and 4.99% Water type. Following the percentage of dominant land use, 53 sites (14%) were categorized as "Urban", 239 sites (65%) as "Agricultural", 71 (19%) as "Natural" and 5 (1.3%) as "Reference". The highest concentration ranges were observed for the "Urban" and "Agriculture" sites, with mean ± one *SD* log[TON] averaging 0.046 ± 0.35 (= antilog 1.60 [1.45 – 1.74] mg/l) and -0.05 ± 0.33 (= antilog 1.22 [1.16 – 1.27] mg/l) respectively. The "Natural" type sites averaged lower means of -0.14 ± 0.28 (= antilog 0.93 [0.86 – 0.99] mg/l) and the "Reference" type sites showed lowest concentrations with -0.17 ± 0.28 (= antilog 0.83 [0.68 – 0.98] mg/l). The higher concentrations were thus observed from sites surrounded by developed land use types (Urban and Agriculture), highlighting the influence of human activities on organic nitrogen runoffs (Figure 11).

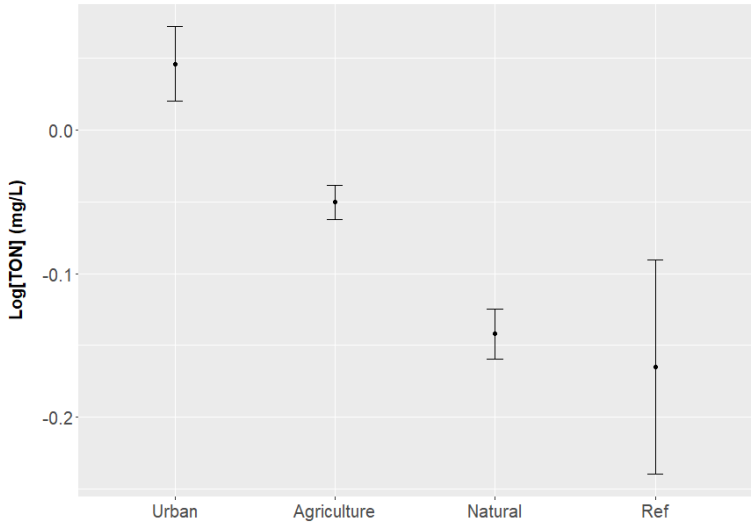

Figure 10 *Mean (points) ± 95% confidence interval of measured log-transformed TON concentrations (mg/L) and classified per dominant land use types.*

IV.2.3 TON trends

The log[TON] anomaly relative to the reference year 2001 decreased from 1990 to 2012 (R^2 = 0.78, p <= 0.001), with a linear trendline showing a decrease of 0.3 log units (-50% in absolute TON) during the period (Figure 12). All the dominant land use classes followed the same observation, with decreasing trends observed regardless of the type at different gradient nevertheless. The "Urban" sites showed the highest rate from 1990 to 2012 (R^2 = 0.77, p <= 0.001), with a linear trendline decreasing by 0.45 log units (-

64% in absolute TON). The dominant "Agriculture" type also showed a decreasing anomaly from 1990 to 2012 (R^2 = 0.75, p = <= 0.001), with a linear trendline decreasing by 0.27 log units (-47% in absolute TON). The "Natural" sites (R^2 = 0.69, p <= 0.001) showed a linear trendline decreasing by 0.22 log units (-40% in absolute TON). The "Reference" sites followed same pattern with an anomaly strongly decreasing (R^2 = 0.30, p = 0.0066) and a linear trendline losing 0.30 log units (-50% in absolute TON). All the graphics for TON trend analysis categorized per land use types are provided as appendices (Figure A-9 to A-12).

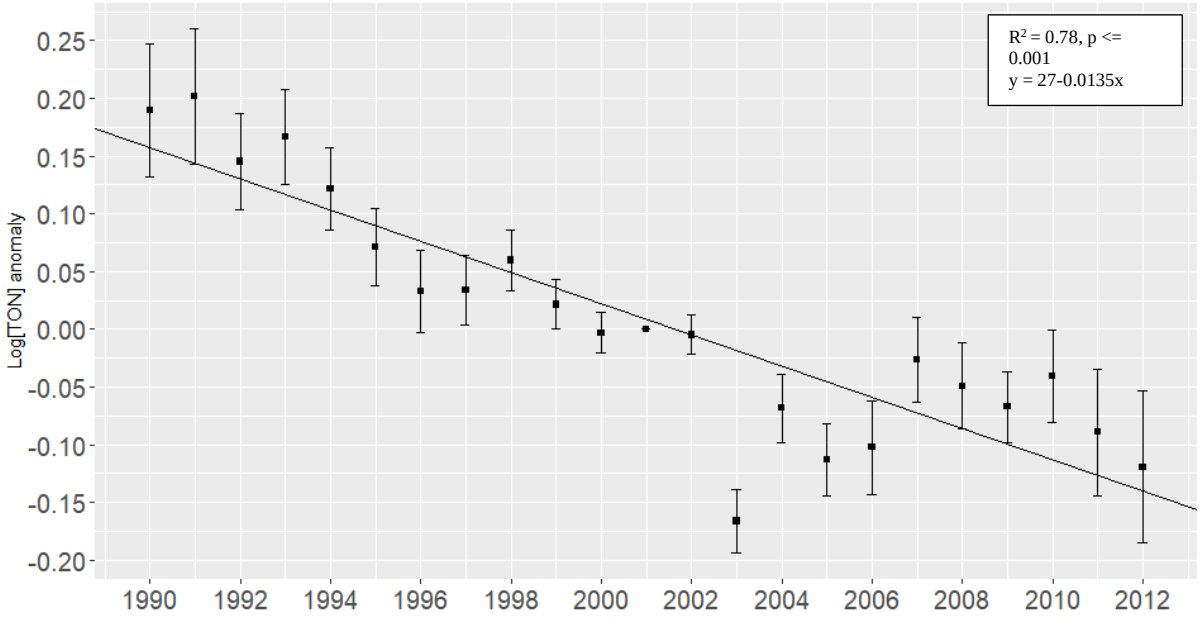

Figure 11 *Anomaly relative to 2001 in log-transformed values of TON per monitoring sites. Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

IV.2.4 TON annual difference and rate of change

For all sites *n* from subset TON (2) excluding the dominant land use "Water" (*n* = 1550), the annually averaged TON concentration difference was -0.01 ± 0.17 mg/l/year, and the annual rate of change averaged 13.41 ± 54.24 %/year. A total of 877 sites showed negative annually averaged differences, for 669 sites showing positive differences and 4 without variations. When classified into dominant land use, "Urban" and "Agriculture" sites showed the lowest averaged concentration difference with -0.007 ± 0.246 [-0.055 – 0.016] mg/l/year and -0.015 ± 0.160 [-0.051 – 0.046] mg/l/year respectively. The "Natural" sites showed rates of 0.004 ± 0.133 [-0.025 – 0.0245] mg/l/year and "Reference" sites averaged the highest difference with 0.038 ± 0.2 [-

0.014 – 0.011] mg/l/year. Of all the sites, 224 showed significant increase (>= 25%/year) and 32 showed a significant decrease (<= -25%/year). The sites showing significantly changing concentrations are charted with bigger symbols in Figure 12.

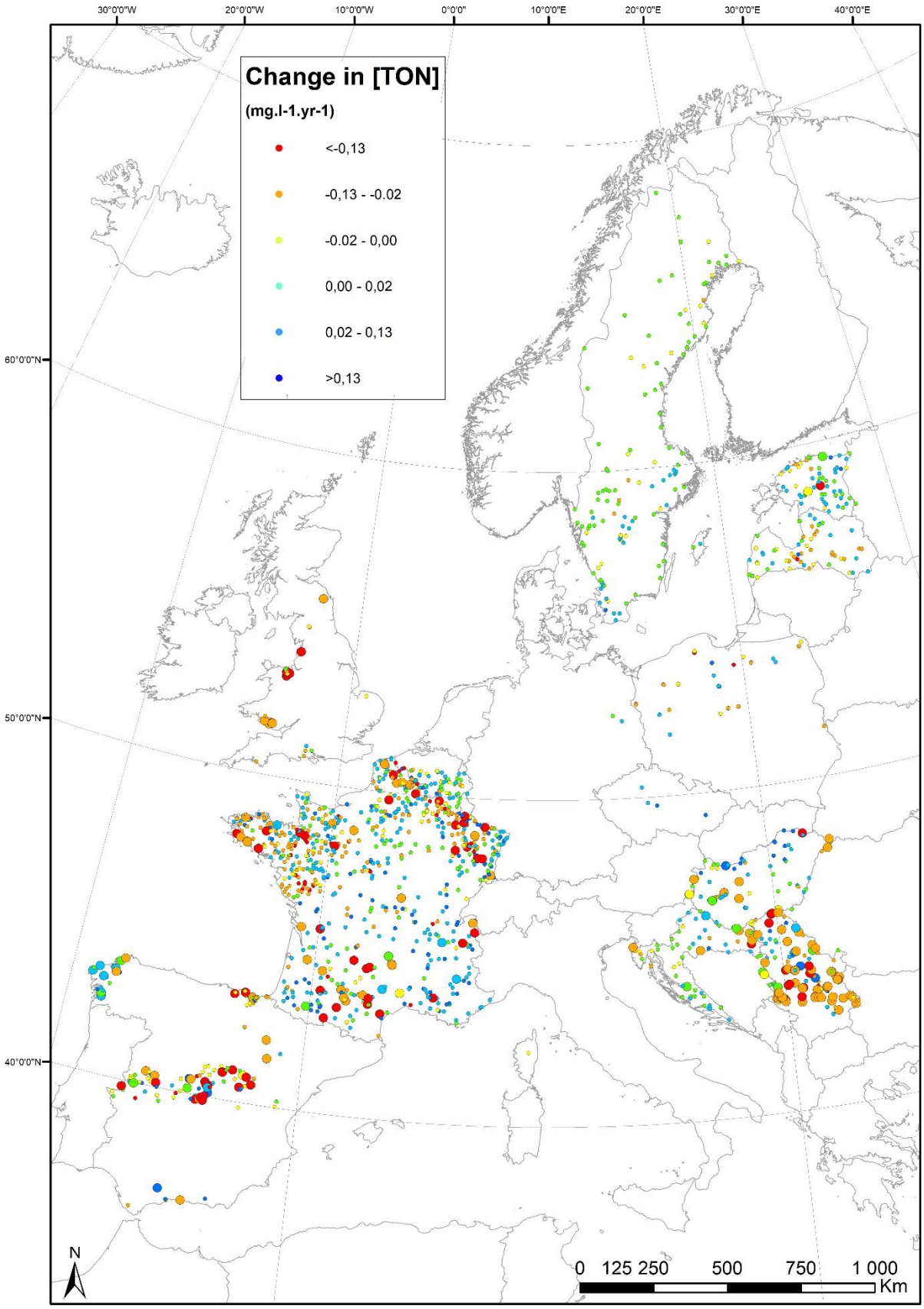

Figure 12 *Trends in total organic nitrogen (mg l-1 yr-1). Symbol size varies with the yearly averaged concentration rate of change (+/- 25%). Data are shown for monitoring sites from the Waterbase showing a minimum of three occurences in annual mean TOC*

IV.3 Relationship between TOC and TON

The mean TOC/TON ratio for all monitoring sites except the "Water" type (n = 505) was 17.10 ± 13.16, with a 95% confidence interval of 16.62 - 17.58 and a 1st to 3rd quartile interval of 6.96 – 24.35. Table 6 shows all the statistics for the TOC and TON annual mean concentrations as well as the TOC/TON ratio. Graphical representations of the datasets' distributions illustrate difference in concentrations, with TOC showing higher concentration values than TON overall. The TON dataset shows concentrations less dispersed than TOC, with 1st and 3rd quartiles more centered on the mean (Figure 13).

Table 6 *Statistics on the annual mean TOC and TON concentrations (mg/L) from the dataset created for TOC/TON study*

Variables	Minimum	1st. Qu	Median	Mean	3rd Quartile	Maximum
TOC	0.5025	3.6721	6.4395	8.2469	11.7000	88.4750
TON	0.0178	0.2904	0.5260	0.7083	0.8507	16.5466
TOC/TON	0.6212	6.9648	14.0575	17.0851	24.3489	164.4443

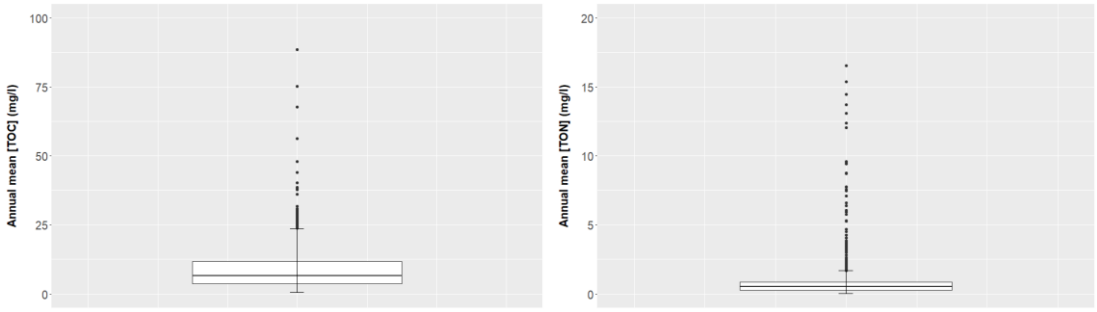

Figure 13 *Box and whisker plots summarizing the distribution of annual mean concentration records (mg/l) for the a) TOC and b) TON from the TOC/TON dataset. Boxes show the median values +/- one quartile, whiskers show min/max values within 1.5 times interquartile range below/above quartiles and points show outliers*

The TOC/TON ratio showed similar patterns for all dominant LU types, with "Urban" sites averaging 14.4 ± 8.72, "Agricultural" sites averaging 14.1 ± 10.6, "Natural" sites 18.7 ± 10.5 and "Reference" sites 26.4 ± 16.7 (Figure 14). Considering the averaged approximations (DOC = 0.9*TOC, DON = 0.7*TON) for European rivers and estuaries, the mean ratio of DOC/DON would be 22.00 ± 16.88, with a 95% confidence interval of 21.37 – 22.61 and a 1st to 3rd quartile interval of 8.99 – 31.32. When considering all the sites without categorization per land use types, TOC and TON concentrations showed a clear correlation with R^2=0.24 (p <= 0.001) (Figure 15). When categorized per land use types, developed land use types such as Urban and Agriculture showed similar results with R^2 of 0.23 and 0.22 (p <= 0.001) respectively. Monitoring

sites with Natural and Reference dominant land use showed higher correlation with R^2 of 0.26 and 0.40 respectively ($p <= 0.001$). All the scatter plot categorized per land use types are provided as appendices (Figure A-13 to A-14). The rate of change of the TOC/TON ratio was calculated for each sites and charted to highlight any distribution patterns in the data (Figure 16).

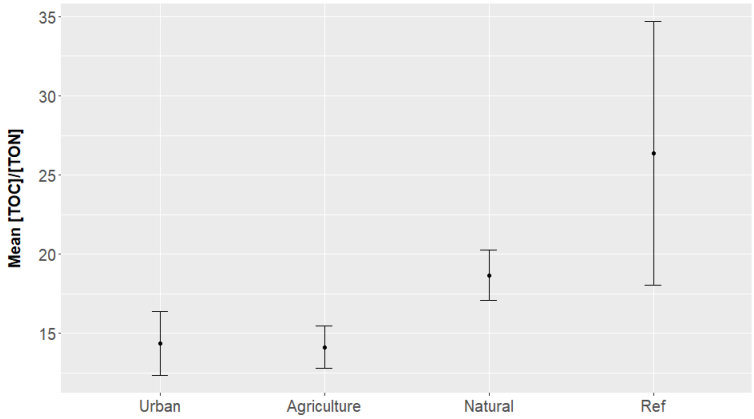

Figure 14 *Mean (points) ± 95% confidence interval of the [TOC]/[TON] ratio averaged per monitorings sites and classified per dominant land use types*

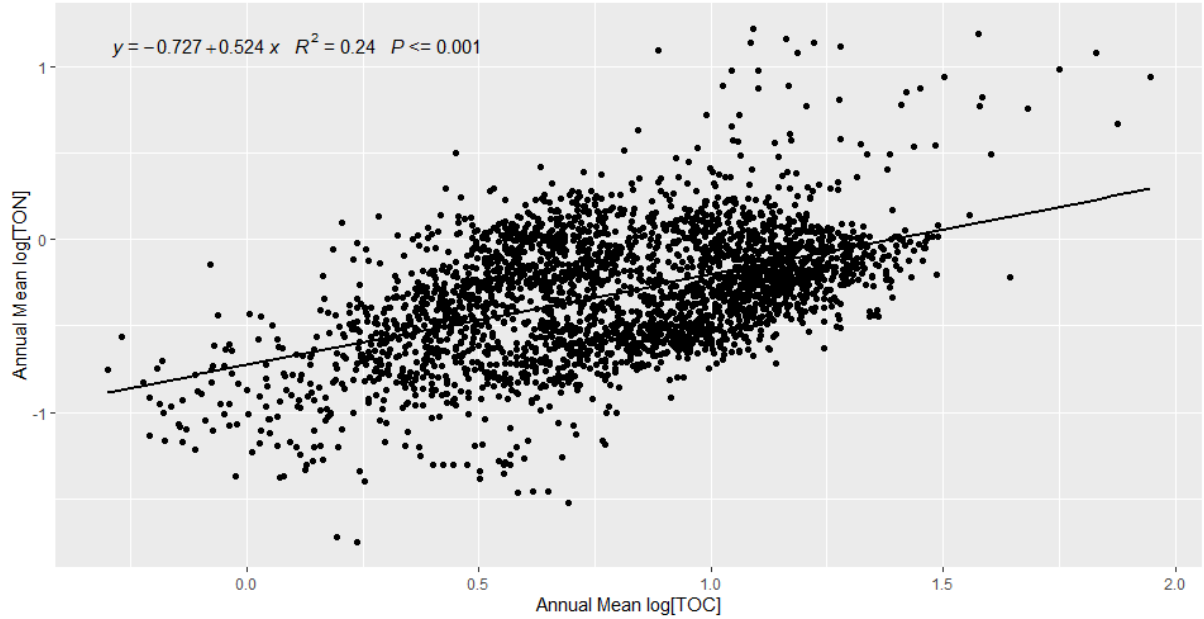

Figure 15 *Scatter plot of annual mean log[TON] relative to log[TOC]. Data are shown from monitoring sites in Europe with a pair of measurements on common years.*

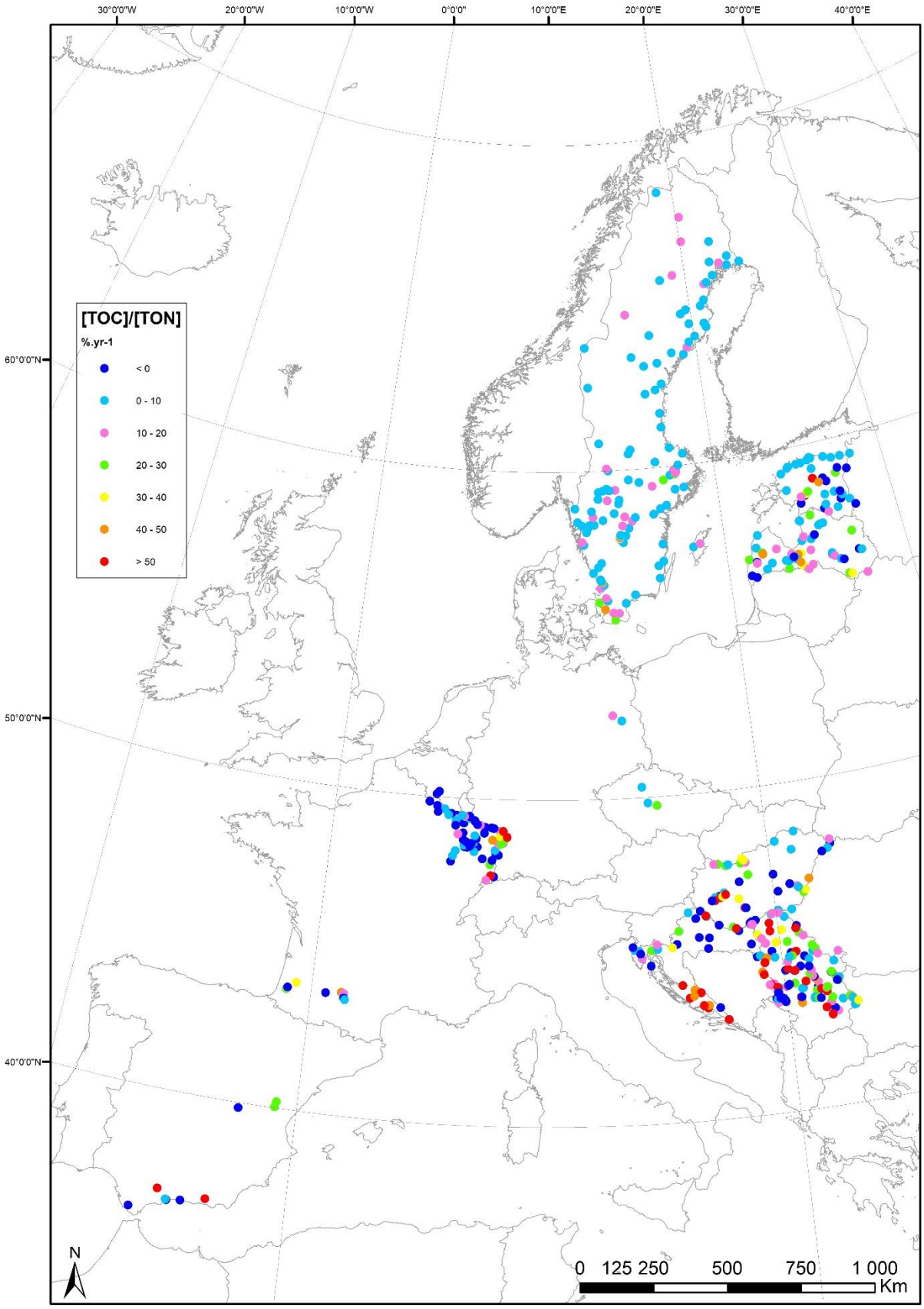

Figure 16 *Trends in TOC/TON ratio (%/year). Data are shown for all monitoring sites from the Waterbase showing TOC and TON measurements on common years.*

V. Discussion

Variations of organic carbon and organic nitrogen concentrations in surface waters reflect the effects of human activities on water ecosystems. The spatio-temporal variations of TOC and TON concentrations sampled from European water bodies are studied here and their trends are correlated to the surrounding dominant land use.

Both the TOC and TON concentrations observed are qualified high and lie at the upper end of the ranges reported globally for rivers and estuaries. Mean concentration ranges of DOC in the major Mediterranean rivers (Rhone, Po, Ebro) are between 1.61 and 2.62 mg/l (Santinelly, 2015). The highest DOC values reported for the Wanquan River (China) averaged 2.33 ± 0.30 mg/l (Wu et al., 2013), and the average global river DOC concentration is estimated to be 5.29 ± 0.22 mg/l (Dai et al., 2012). This comparison with global DOC ranges was made by using a conversion factor observed globally (DOC = 0.9 * TOC) and comforted by results obtained from a similar dataset (Berggren & Al-Kharusi, 2020). Nevertheless, comparison between the Waterbase and global observations should be taken with care as the morphology and sizes of the streams are not considered. In fact, records from the Waterbase are assumed to origin from smaller rivers rather than large global streams, which can lead to and explain much higher concentrations. On the other hand, the organic matter is degraded over time as it flows through the aquatic network, and groundwater input contributes to further diluting the concentrations in large rivers. As methodology improvement, it is suggested to categorize the concentrations of organic C and N based on the morphology of their recipients, and to further compare results from different datasets based on water body morphologies.

It has been observed that DON generally averages 58-77% of the TDN pool within lakes, rivers, estuarine, and surface ocean waters (Sipler and Bronk, 2015; Xia et al., 2018). A study made on approximately 1000 Norwegian lakes confirms these figures by averaging a TN fraction made of 71% DON, reflecting that organic N is an important part of TN (Hessen et al., 2009). Considering this approximation, the mean concentrations calculated from the Waterbase are consistent with global DON observations and lie on the upper end of the ranges observed globally for estuaries (0.303 ± 0.223 mg/l) and rivers (0.333 ± 0.254 mg/l) (Sipler and Bronk, 2015). This

study did not focus on the dissolved organic fraction part of total organic fraction, but ratios documented for both C and N reinforce the view that omission of the dissolved organic measurements in water quality assessments may result in an underestimation of the inputs of total organic fraction in water systems and subsequently underestimate eutrophication pressures. Consequently, the calculation of the dissolved organic fraction relative to total organic fraction should be systematically added to the methodology as lead for improvement.

A summarized TOC concentration calculated from a global dataset of approximately 8300 lakes taken from 68 countries/regions on 6 continents averaged 5.578 ± 2.8 (5.43 – 5.72) mg/l. The lowest mean TOC (3.690 mg/l) was observed from lake water in the north frigid zone and the highest mean TOC (5.809 mg/l) was observed from the south temperate zone, concluding on TOC concentrations unevenly distributed around the world with a climate dependent gradient (Chen et al., 2015). The present study focused on the relationships between TOC and dominant land cover classes, but the wide spatial extent of the analysis made by Chen et al. (2015) suggests a similar gradient to exist in Europe. The number of entries resulting from data filtering is an issue to reach a well distributed dataset over the European continent, but the study of a geographic gradient in Europe would constitute a lead for further research.

The “Reference” sites showed the highest TOC concentrations, highlighting the fact that labile organic compounds (Lapierre et al., 2013) from terrestrial plant sources dominate as TOC source in many freshwaters (Wilkinson et al., 2013). However, these results are oppositely different from Berggren and Al-Kharusi (2020), who observed higher TOC concentrations from Urban and Natural sites. This could be explained by different data preparation, calculation methodologies or the categorization of the water bodies from the Waterbase. The “Reference” sites studied are represented by two water body categories: Lake water (n = 433) and River water (n = 565). As listed in Table 2, the definitions for the water body categories published by the EEA (Directive 2000/60/EC) are broad and do not inform on the morphology on the units. Considering that the “Reference” class is attributed to a monitoring site surrounded by more than 95% “Natural” land use, the high concentration of TOC could be assumed for small water bodies (e.g. small streams) surrounded by dense forest environment. A better categorization of the land cover by the mean of remote sensing and associated to a better

identification of the water body morphologies (e.g. stream widths) could help refining the results for further studies.

The highest TON concentrations were observed from "Urban" and "Agricultural" dominant sites (Figure 11), reflecting the effects of developed land parcels as important source of DON in rivers and streams. Both autochthonous biological processes and anthropogenic organic matters produced by human activities on land contributes as DON sources in marine systems (Keller and Hood, 2011; Lonborg et al., 2009; Miller, 1999). Linked to human activities, it has been reported that treated wastewaters are an important source of DON in urbanized rivers and streams (Kalscheur et al., 2012; Meng et al.; 2013), from animal feedlots and storage lagoons (Sun et al., 2017) amongst others. High surface water DON concentrations have also been linked to runoffs from intensive agricultural activities in the United States (Westerhoff and Mash, 2002). Seitzinger et al. (2002) reported that a higher proportion of human activity derived DON was bioavailable to marine ecosystems relative to forest-derived DON, meaning that urban and agriculture activities not only alter the concentrations of DON in hydrologic N losses but also have implications in understanding nutrient over-enrichment and eutrophication episodes in estuarine and coastal systems.

Estuarine and coastal systems are among the most productive ecosystems on Earth (Odum 1971), where nutrient over-enrichment creates a consequent stress that can lead to the development of eutrophic conditions in severe cases (Driscoll et al., 2003). Nitrogen is the most critical element in coastal ecosystems (Ryther and Dunstan, 1971; Oviatt et al., 1995), on the opposite of freshwater systems where eutrophication is mainly caused by excess of phosphorus (Vollenweider, 1976). Coastal eutrophication can be responsible for excessive production of algal biomass, blooms of toxic algal species, loss of important estuarine habitat, changes in marine biodiversity and species composition, increases in sedimentation of organic particles, and depletion of dissolved oxygen (hypoxia and anoxia). These primary effects can have further impacts on the food web (e.g. effects of hypoxia on fish) (Driscoll et al., 2003). Comprehensive and global datasets documenting the frequency, extent and rate of over-enrichment episodes are unfortunately lacking but national collections are being more and more published. The Waterbase adds to this dynamic and the results observed for decreasing TON concentrations may lead to an improvement of the effects listed above. We have a

limited understanding on the capacity of estuaries and coastal systems to recover from decreasing nutrient inputs, both in rate and extent. It is thought that systems dominated by phytoplankton with short water residence time will reverse their eutrophication trajectories faster. On the other hand, benthic-dominated systems with rooted, submerged aquatic vegetation will show delayed recovery (Driscoll et al., 2003).

The TOC concentrations showed an increasing trend from 1990 to 2012, confirming the observations made globally on the European continent (R^2 = 0.28 for log[TOC] anomaly from 1994 to 2014, Berggren and Al-Kharusi 2020) and North America. Many hypotheses, such as climate changes enhanced by human activity (Worrall et al., 2003; Freeman et al., 2001), nitrogen deposition (Findlay, 2005) or changes in land use (Garnett et al., 2000) have been published to explain unprecedented levels of TOC that will continue to rise in the future. Alternatively, other studies suggested that DOC levels are in fact returning toward pre-industrial levels as a result of gradual decline in the sulphate content of atmospheric deposition (Evans et al., 2005; Stoddard et al., 2003; Vuorenmaa et al., 2006). Monteith et al. (2007) published new results to explain declining DOC trends as a result of simultaneous fall in atmospheric sulfur SO_4^{2-} and chloride Cl^-.

The concentrations around "Urban" (Figure A-5) and "Agricultural" (Figure A-6) sites showed trends decreasing slightly, reflecting improvements made in wastewater treatment and mitigation measures to reduce organic matter imports from agricultural fields (EEA, 2015; Skjelkvale et al., 2005). The "Natural" (Figure A-7) and "Reference" (Figure A-8) sites showed steep increase over the same time range, confirming the dominance of natural land cover in the import of labile terrestrial organic matter observed previously. Additionally, it has been observed that the decay coefficient k decreased by up to 50% from 1996 to 2012 in hundreds of rivers from the European continent (Berggren and Al-Kharusi, 2020). This could be an indicator of TOC source shifting from internal production by algae to external loading from terrestrial environment in surface waters. Other results stand as indicators to support this shift in TOC quality and source, such as a stable or decreasing levels of total and inorganic N and P from similar continental study in Europe (EEA, 2015) and general increases in colored terrestrially derived organic carbon in European freshwater (Hejzlar et al., 2003; Hruska et al., 2009; Montheith et al., 2007; Skjelkvale et al., 2005).

The opposite observation was made on TON concentrations, which showed a decreasing trend regardless of the land use classification. The Urban (Figure A-9) and Agricultural (Figure A-10) sites showed the highest R^2 coefficients, following the previous observations on the contribution of land-derived anthropogenic organic matter to the levels of DON in surface waters. European rivers have experienced improved water quality over the last decade, with decreasing concentrations of N and P in response to reduced use of excessive fertilizers in agriculture and better wastewater treatment (EEA, 2015, 2018).

Concentration trends are the result of permissive data filtering and should be handled with care. A strict filtering of sites with a minimum of observations within the time range of interest could be applied, as done by Monteith et al. (2007) for example. The number of observations should also be subject to other criteria, such as a minimum number of monthly observations over the time range of study or a minimum of observations to aggregated a yearly average for example. When this is not an issue for TOC observations, the interest given to organic N monitoring is relatively recent (Evans et al., 2005) and statistical calculations can be influenced by too few observations.

The TOC:TON ratio showed high values compared to the ranges observed globally for rivers (32.5 ± 16.3) and estuaries (16.0 ± 7.40), regardless on the type of dominant land cover (Sipler and Bonk, 2015). The comparison to ranges observed worldwide was made by approximating the DOC and DON concentrations with averaged factors observed. All land cover types showed proportional increases between N and C, with ratio ranging from 0.25 to 0.40 in favor of C. This highest R^2 was observed for "Reference" sites (Figure A-16), highlighting again the dominance of surrounding natural landscapes for TOC inputs. The global pattern observed of N and C increasing at different ratios confirms that organic N is closely associated with TOC, both originating primarily from allochthonous DOM (Kortelainen et al., 2006a) and that TOC, TN and TP are exported together from the terrestrial environment (Chen et al., 2016).

The evolution of TOC:TON ratio towards higher values informs on the organic matter quality and their derivate sources. As explained by Berggren and Al-Kahrusi (2020),

organic matter showing low C:N ratio is highly biodegradable (Fellman et al., 2009; Islam et al., 2019), because of its low contents of recalcitrant aromatic carbon rings (Hood et al., 2005), but high content of aliphatic and peptide-like structures (Kellerman et al., 2018; Textor et al., 2019). High organic N contents is common in agriculture runoffs (Textor et al., 2019) and typically originates from labile benthic algal and phytoplankton sources, whereas low N contents (high C:N) is linked to detritus from terrestrial sources (Balakrishna & Probst, 2005; Kaiser et al., 2004). Most allochthonous plant detritus entering rivers has C:N > 20, but the variability is large and some terrestrial organic matter sources such as fens, marshes, or sewage have lower ratios (Fellman et al., 2009; Lehman et al., 2015; Vaquer-Sunyer et al., 2016). Another explanation for high TOC:TON ratio could be the phytoplankton growth as a main source of C-enriched DOM (carbohydrate), as observed in the West Neck Bay, Shelter Island (Myklestad, 2000). Analysis from the same study showed that high molecular weight (HMW) organic matter was enriched in phytoplankton-derived carbohydrates and that such fresh DOM has often high C/N ratio. This large variability of C:N ratios that exists from both natural and human organic matter sources could explain why no overall nor distinct relationship with land use could be found.

This study was designed to highlight global trends relative to dominant land cover categories for straightforward output. Studying the evolution of variables' concentrations relative to the percentage of land use category in the vicinity, to apprehend linearity and threshold values such as applied by Herlihy and Stoddar (1998), could constitute a lead for methodology improvement. Suggestion are also made to study trends per water body types, as the geomorphology influences the spatiotemporal evolution of variables within and are exposed to climatic events. The example given is recipient lakes standing as "sentinel" for the study of N within catchments (Hessen et al., 2009).

The calculation of the land cover fraction around sites and the resulting classification into dominant types were made empirically within a buffer of 1km. This method was preferred to fit the wide spatiotemporal extent of the original database and the scope for outputting global trends. As integration improvement, land uses should be selected within watersheds and related to geo-variables such as slopes, geology, population density or local precipitation for example. Another suggestion would be to study

chemical compounds' trends against land use changes in time. The land use dataset used here depicts a situation at a given time, without indication of changes over time. For example, Hong et al. (2011) presented a methodology to calculate the area change rate for each land cover classes between land cover datasets. The rates are then assigned to a raster and easily integrated for correlation analysis with chemical variables' trends (Meneses et al., 2015).

VI. Conclusion

Based on a large database containing 3.2 million records of water quality indicators from monitoring sites distributed over the European continent, we were able to study the concentration ranges and trends of organic C, organic N and consequent TOC/TON ratio from 1990 to 2012. Furthermore, the results stated are correlated to the land cover surrounding each monitoring sites within a radius of 1 kilometer. We show that both the TOC and TON concentrations observed are qualified high when compared to concentration ranges observed globally for rivers and estuaries. The highest TOC concentrations are observed from monitoring sites categorized as "Reference site" (surrounded by >95% natural land cover), highlighting the role of surrounding terrestrial plants and dense vegetation parcels as sources of organic matter in freshwaters. On the other hand, the highest TON concentrations are observed from sites categorized as "Urban" and "Agricultural", depicting the effects of surrounding human activities on surface water quality. The TOC:TON ratios observed are also qualified high when compared to global ranges, regardless of the surrounding land cover. A strong positive correlation is systematically observed between organic N and organic C, with ratio ranging from 0.25 to 0.40 in favor of organic C.

The TOC concentrations showed an increasing trend overall, from 1990 to 2012. When categorizing the sites per surrounding land cover, the "Urban" and "Agricultural" sites showed slightly decreasing trends whereas "Natural" and "Reference" sites showed steeply increasing trends. The TON concentrations showed the opposite behavior, with an overall decreasing trend from 1990 to 2012. When categorized per land cover types, all TON monitoring sites showed decreasing trends, with the "Urban" and "Agricultural" trends showing the strongest R_2 coefficient. This follows the observation made of decreasing N and P concentrations in European freshwaters and highlight the results of improved monitoring methodologies and policies in place in Europe (e.g. reduced use of excessive fertilizers and better wastewater treatments) for an improved surface water quality. As stated above, N is the most critical element for over-enrichment episode in coastal zones. Decreasing organic N concentrations could suggest an improvement in the occurrence of eutrophication episodes in coastal zones and a partial or complete recovery depending on the nature of the ecosystem in place.

VII. Appendices

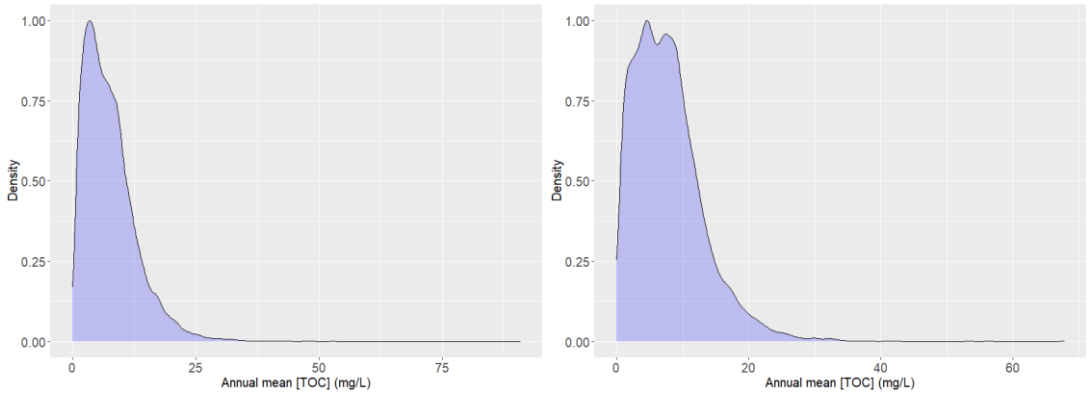

Figure 17 *Distributions of mean concentrations for a) TOC (1) and b) TOC (3)*

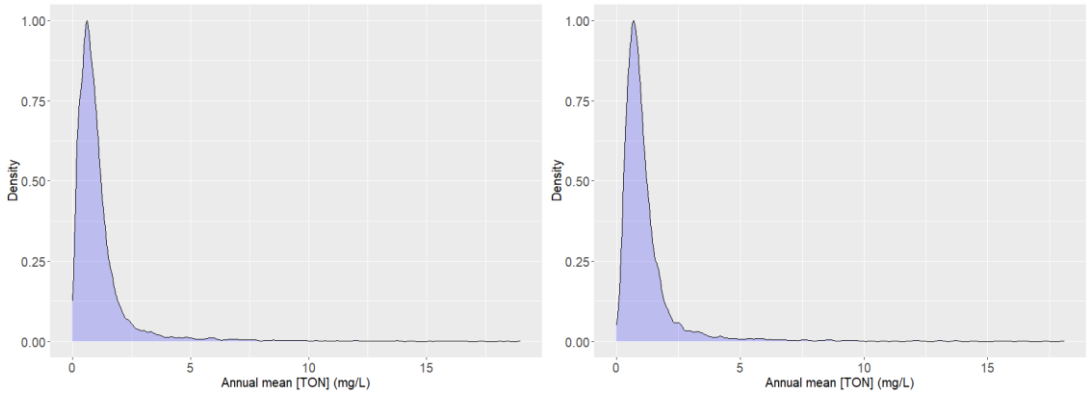

Figure 18 *Normal distributions of mean concentrations for a) TON (2) and b) TON (4)*

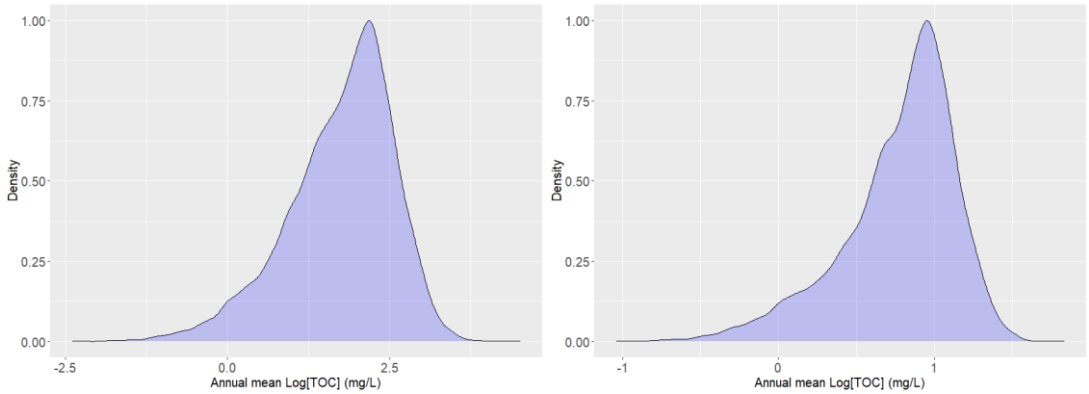

Figure 19 *Log-transformed distributions of mean concentrations for a) TOC (1) and b) TOC (3)*

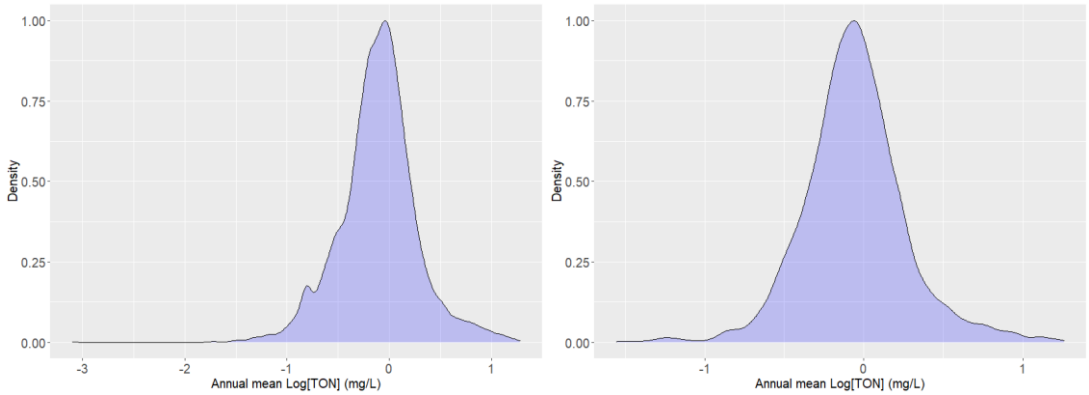

Figure 20 *Log-transformed distributions of mean concentrations for a) TON (2) and b) TON (4)*

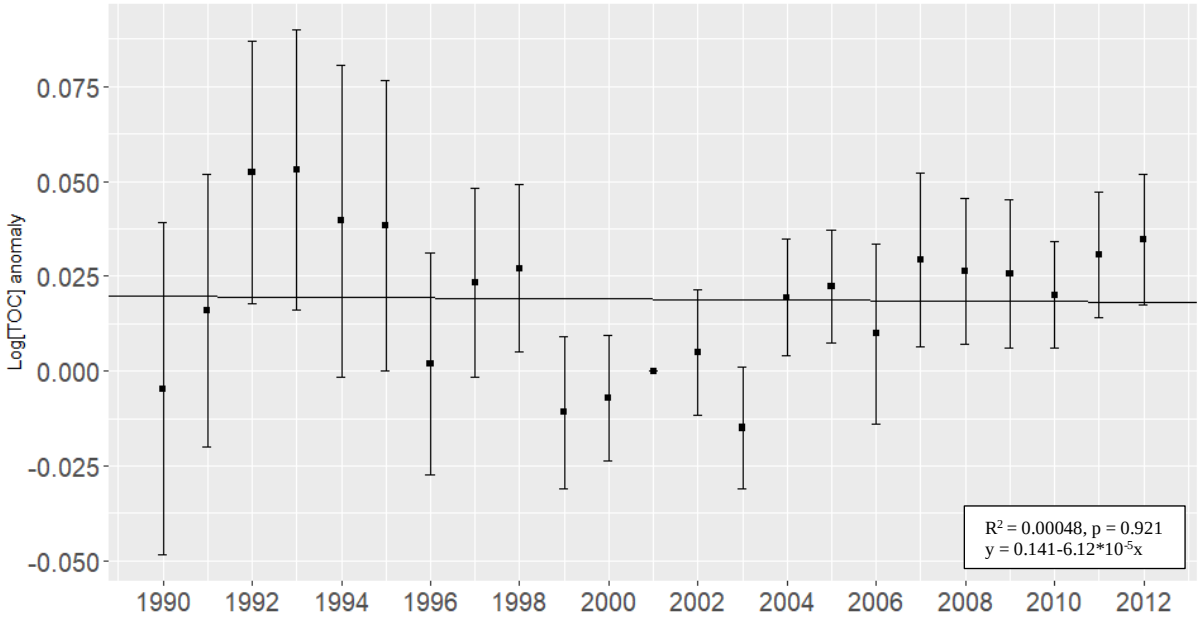

Figure 21 *Anomaly relative to 2001 in log-transformed values of TOC dominant land use type "Urban". Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

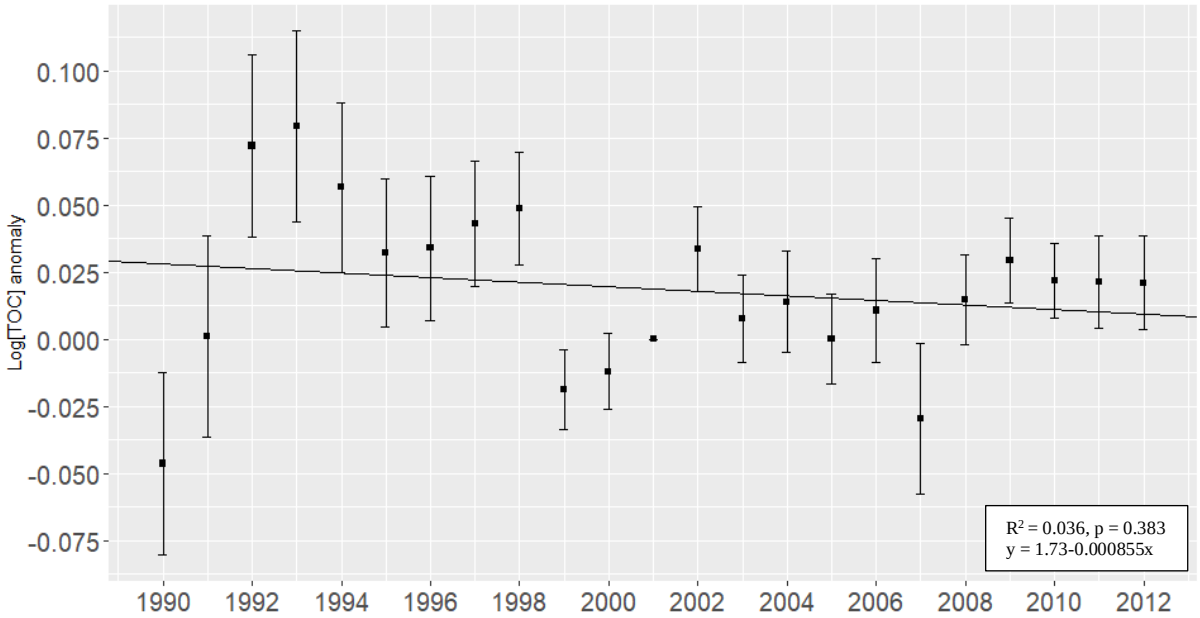

Figure 22 *Anomaly relative to 2001 in log-transformed values of TOC for dominant land use type "Agriculture". Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

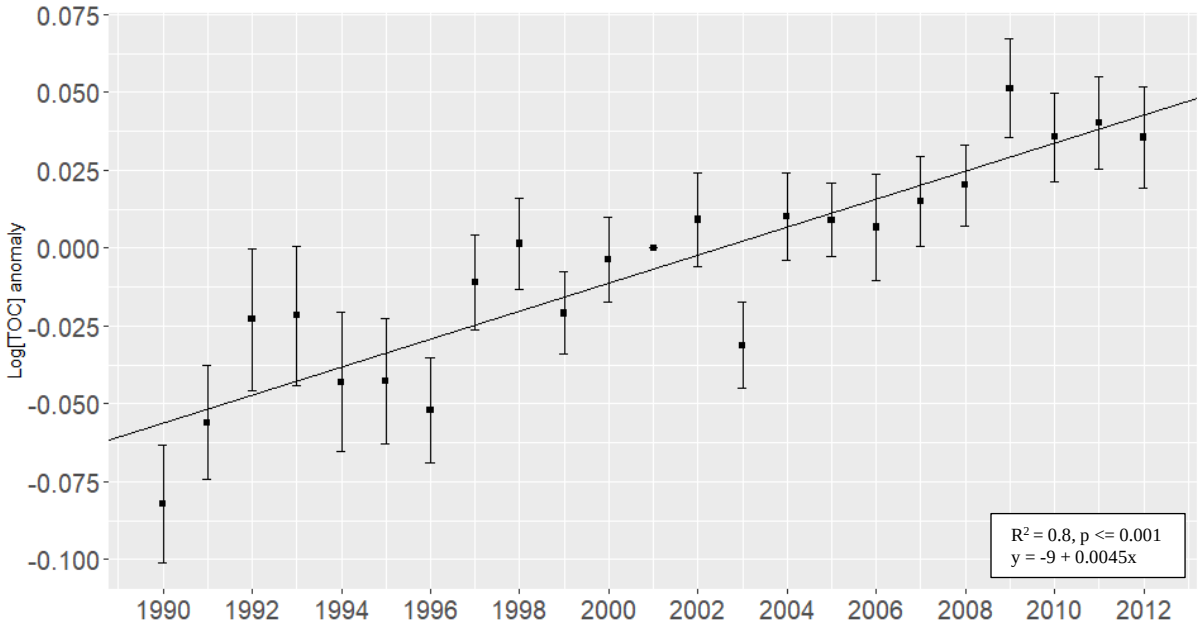

Figure 23 *Anomaly relative to 2001 in log-transformed values of TOC for dominant land use type "Natural". Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

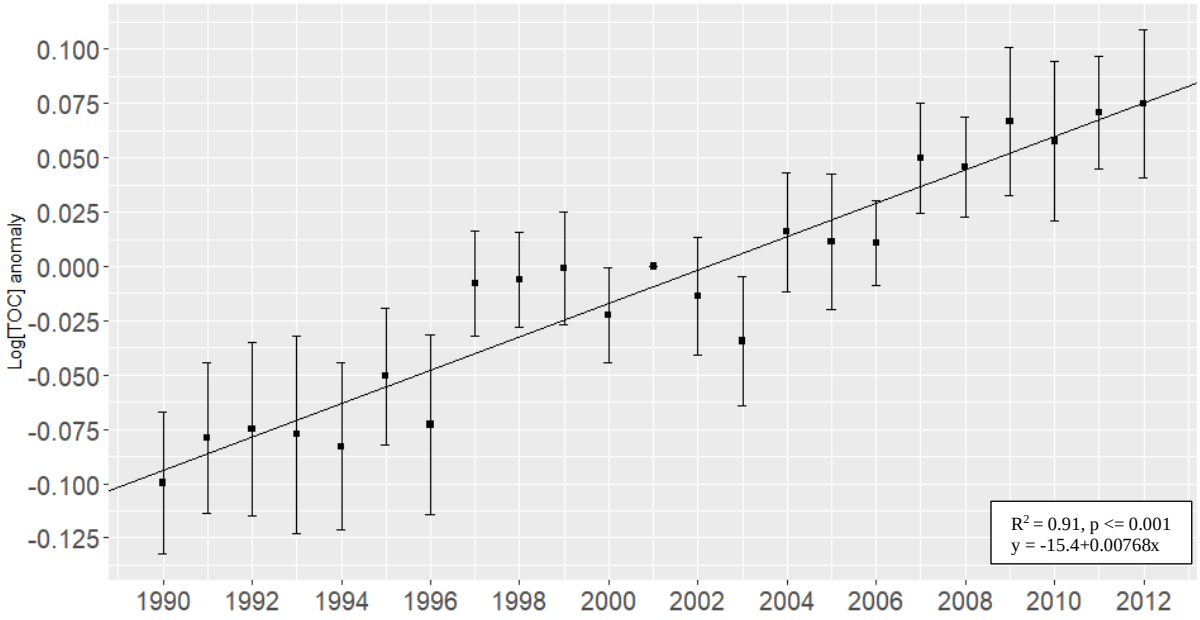

Figure 24 *Anomaly relative to 2001 in log-transformed values of TOC for dominant land use type "Reference". Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

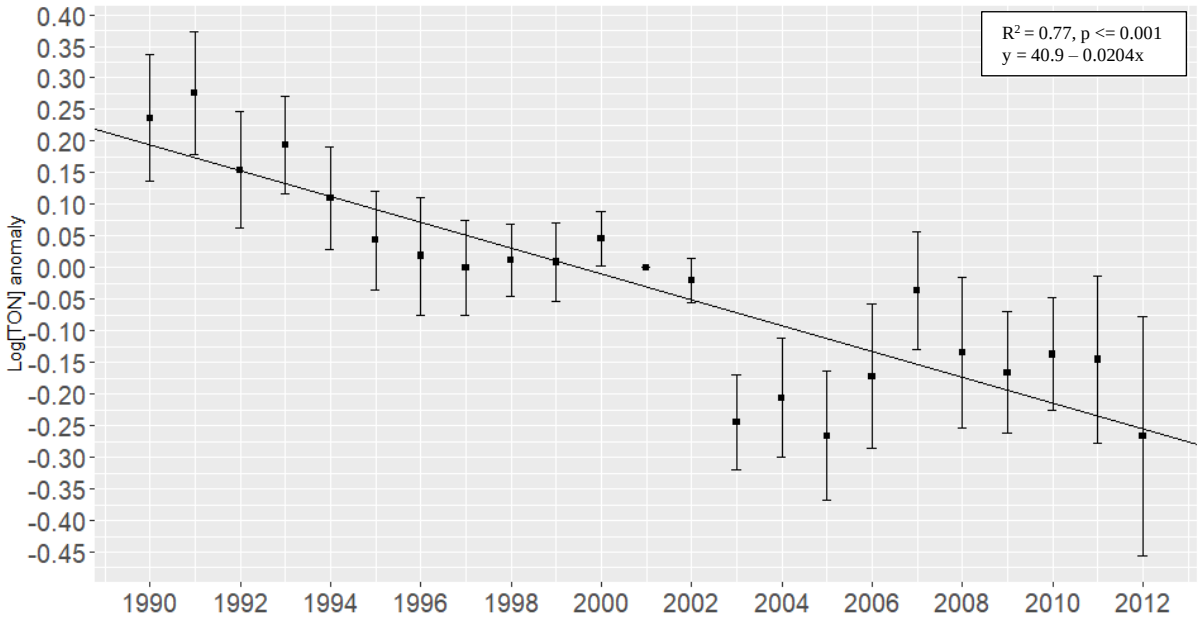

Figure 25 *Anomaly relative to 2001 in log-transformed values of TON for dominant land use type "Urban". Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

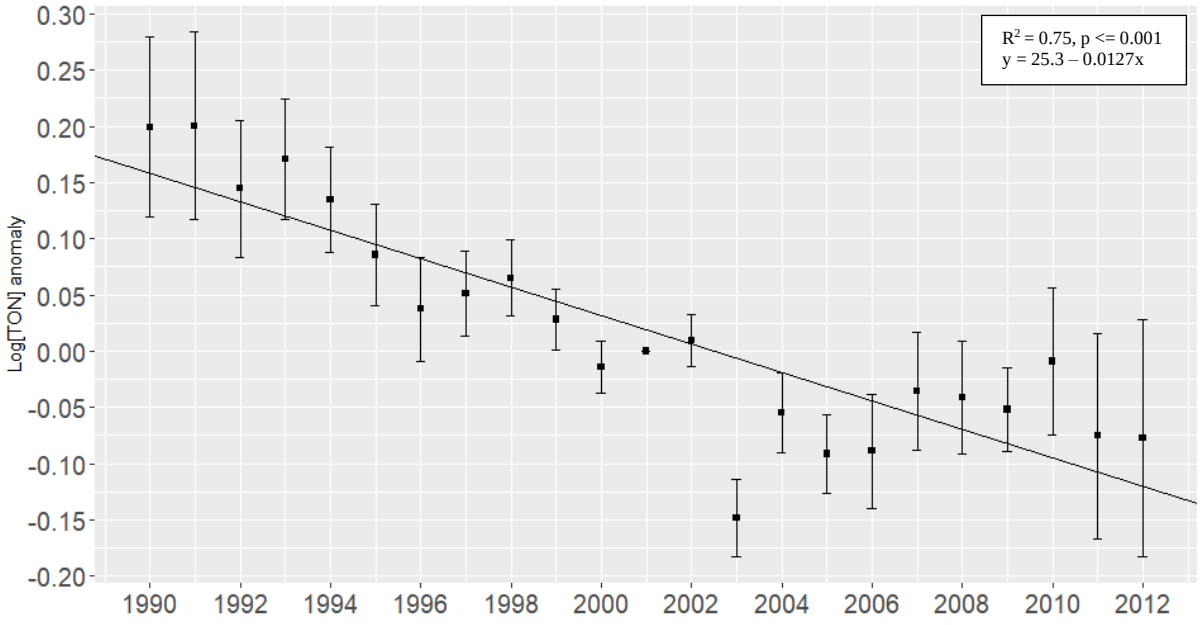

Figure 26 *Anomaly relative to 2001 in log-transformed values of TON for dominant land use type "Agriculture". Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

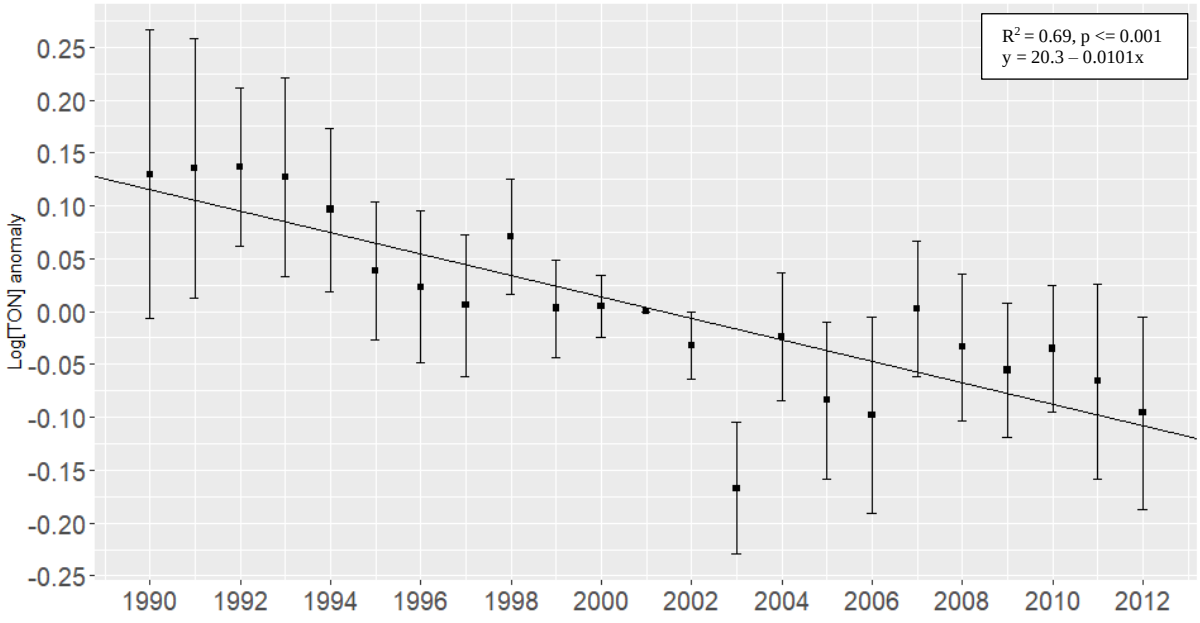

Figure 27 *Anomaly relative to 2001 in log-transformed values of TON for dominant land use type "Natural". Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

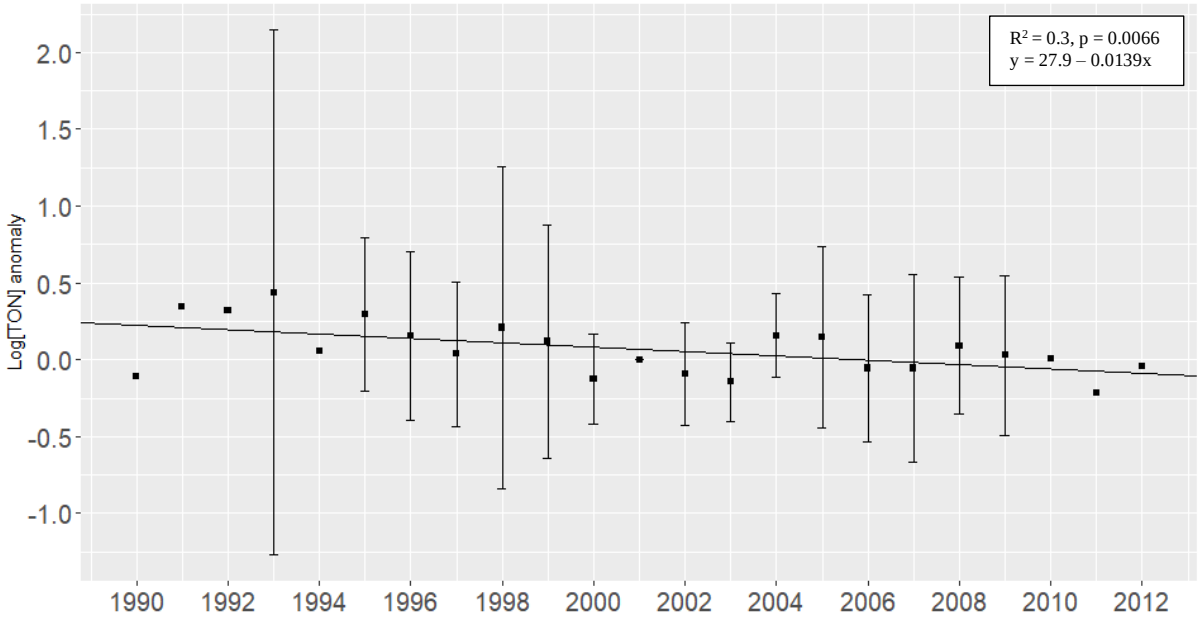

Figure 28 *Anomaly relative to 2001 in log-transformed values of TON for dominant land use type "Reference". Symbols and error bars show the mean and 95% confidence interval of the mean, respectively, of the deviation from 2001 values.*

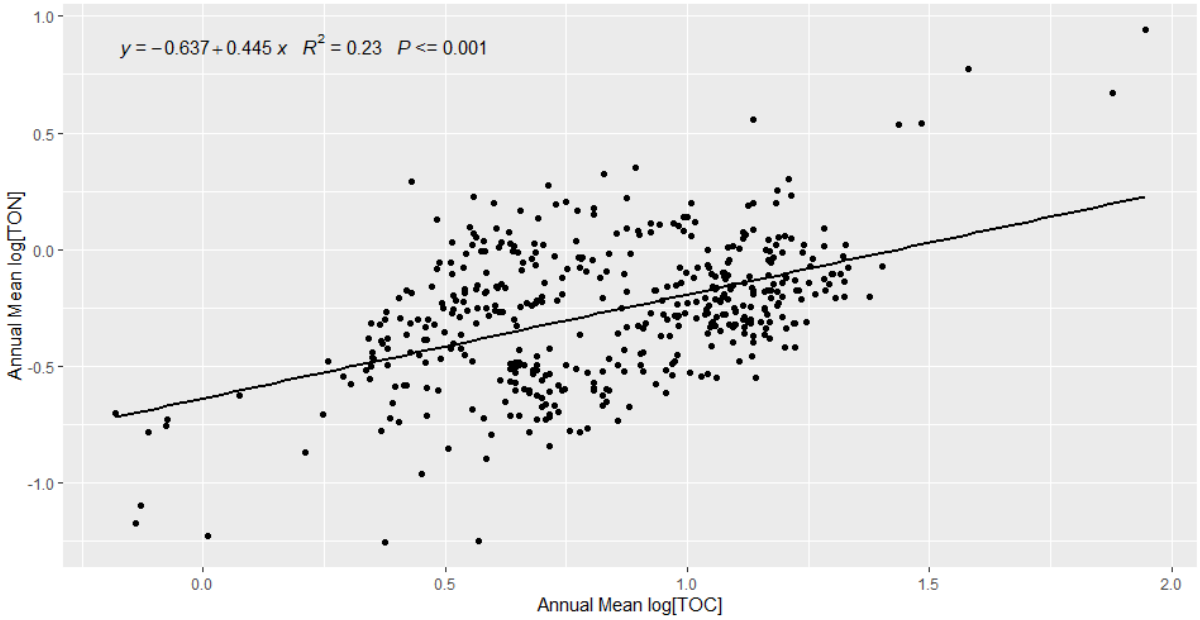

Figure 29 *Scatter plot of annually mean log[TON] relative to log[TOC]. Data are shown from monitoring sites in Europe with a pair of measurements on common years for the "Urban" dominant land use type.*

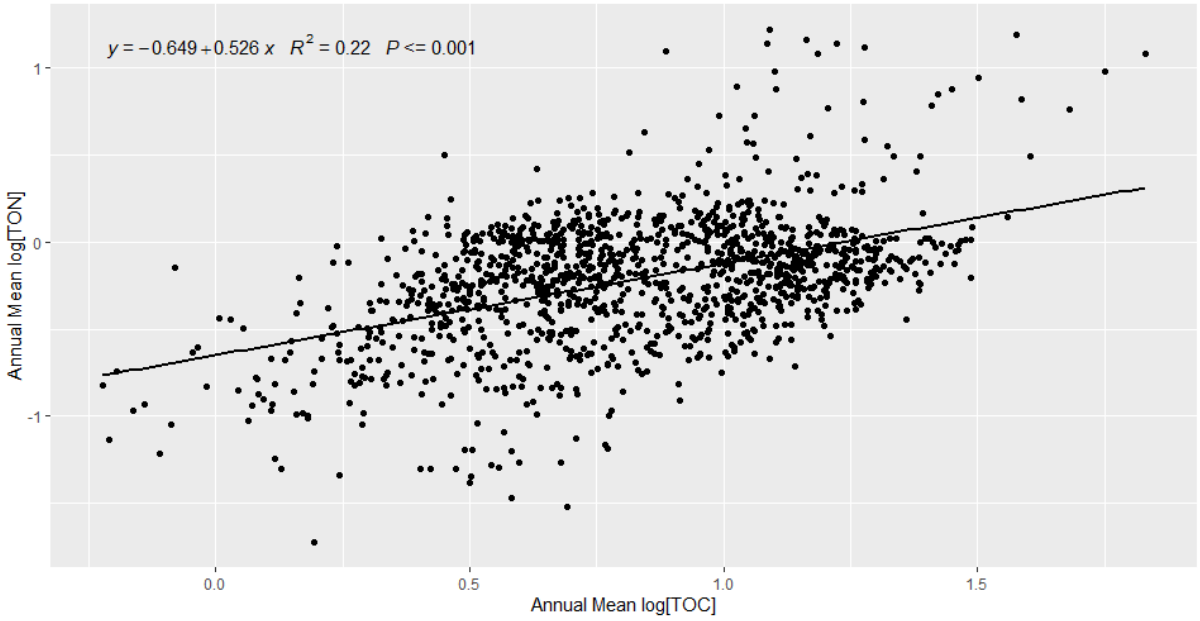

Figure 30 *Scatter plot of annually mean log[TON] relative to log[TOC]. Data are shown from monitoring sites in Europe with a pair of measurements on common years for the "Agriculture" dominant land use type.*

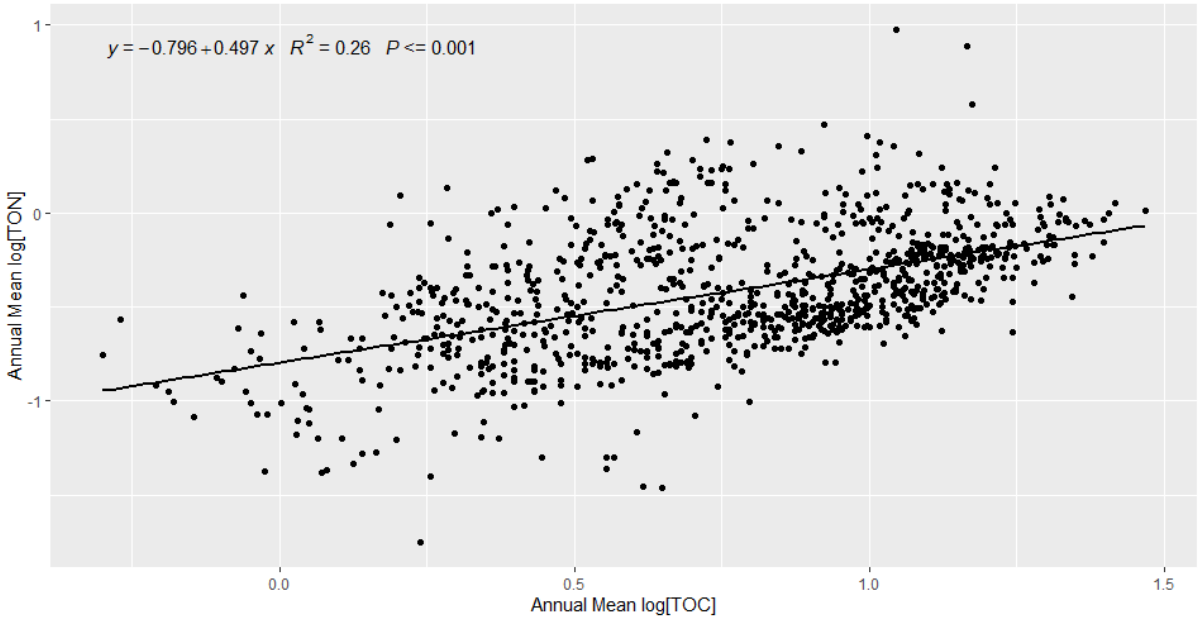

Figure 31 *Scatter plot of annually mean log[TON] relative to log[TOC]. Data are shown from monitoring sites in Europe with a pair of measurements on common years for the "Natural" dominant land use type.*

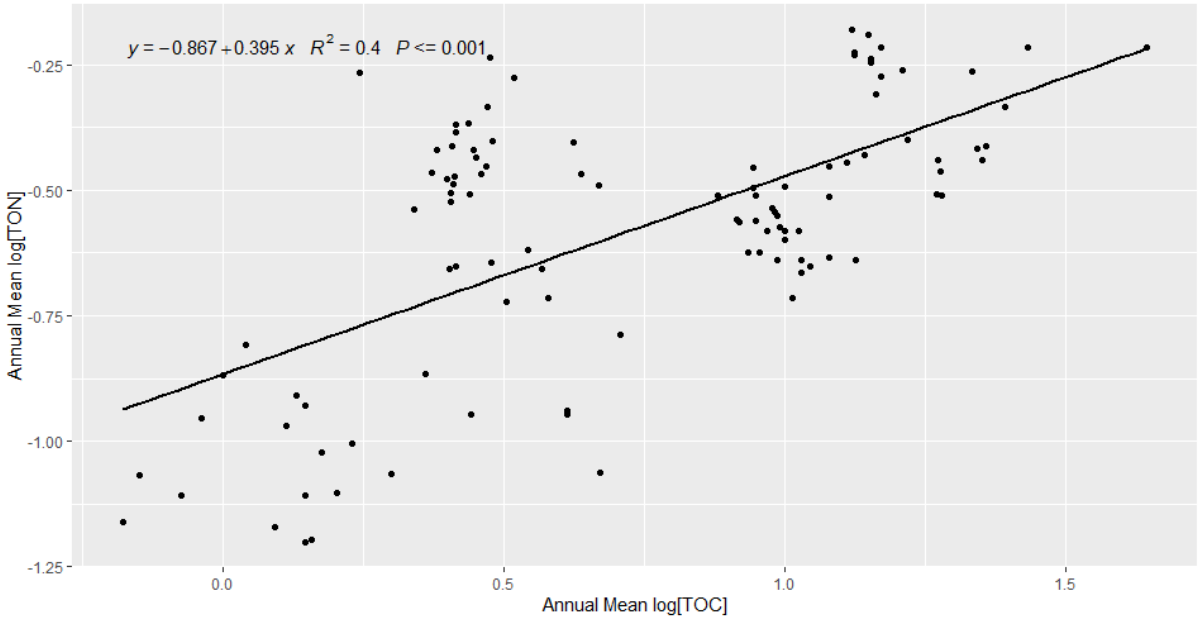

Figure 32 *Scatter plot of annually mean log[TON] relative to log[TOC]. Data are shown from monitoring sites in Europe with a pair of measurements on common years for the "Reference" dominant land use type.*

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