Pesticides and salinisation, two stressors of freshwater ecosystems

by

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SUMMARY

Freshwater is habitat for aquatic biota and supplies essential services for human well-being. However, freshwater, subjected to multiple interacting stressors, is facing quality degradation and deterioration of biodiversity due to hydrological and physical alterations brought about by point source and diffuse pollution. Although it has been known for a long time that anthropogenic activities alter the total concentration pollutants in rivers, there is limited documentation on examining the freshwater micropollutants originating from wastewater treatment plants (WWTPs) on a large spatial scale, how baseline pollutants might change in the future under changing climatic and land-use conditions, how the taxa communities adapt to polluted conditions, and forecast the alteration under climate change. Studies that answer these questions are pivotal for rational and efficient management of freshwater pollution.

This thesis examined two specific cases of point and diffuse pollution, pesticides and salinisation, which are two of the most concerning stressors of Germany's freshwater bodies. The findings of this thesis were organized into three major components, of which the first component presents the contribution of WWTPs to pesticide toxicity (Chapter 2). The second component focuses on the current and future background salt ion concentrations under climate change with the absence of anthropogenic activities (Chapter 3). Finally, the third major component shows the response of invertebrate communities in terms of species turnover to levels of salinity change, considered as a proxy for human-driven salinisation (Chapter 4).

In chapter 1, I present a brief introduction to the spatial extent of pesticides and salinity pollution in freshwater, the causes of two pollutants, occurrence in freshwater and their effects on aquatic organisms. I also give an overview of the research questions, and the aims of the thesis. Chapter 2 reports the contribution of WWTPs to freshwater pesticide toxicity at a large spatial scale. I focused on small agricultural streams, which may be subject to the largest inputs. I found that WWTPs contribute considerably to streams pesticide toxicity. Chapter 3 presents the results of fitting a multiple linear regression with the elastic net and a random forest model to predict dissolved salt ion concentrations, including EC, and the specific ions Ca^{2+}, Mg^{2+}, and SO_4^{2-}, in running water bodies that were subsequently used to forecast the alteration under the climate change in absence of anthropogenic contribution. I forecast an approximately 10 – 15 % increase in average EC in German streams by the end of century. Finally, by applying the Jaccard similarity index for invertebrate data (Chapter 4), I observed major invertebrate turnover when the change in EC (considered as a proxy for human-driven salinisation) exceeded 0.4 mS cm^{-1}. All studies were applied for German freshwaters.

Overall, this thesis shows both point and diffuse sources pollution pose a large-scale threat to surface water in Germany. However, freshwater ecosystems are impacted by a wide range of pollutants with different ratios, potentially leading to interactive effects and obscuring causality between pollutant levels and responses. Therefore, adopting a multiple stressor approach is imperative for an integrated ecological and ecotoxicological assessment of rivers.

ABBREVIATIONS

ATKIS	Authoritative Topographic-Cartographic Information System
CSO	Combined sewer overflow
DEM	Digital elevation model
EC	Electrical conductivity
JI	Jaccard Index
LC_{50}	Lethal Concentration 50
LAWA	Working Group on Water of the German federal states
LR	Linear regression
MCPA	2-methyl-4-chlorophenoxyacetic acid
MSE	Mean squared error (MSE)
mTU	Maximum Toxic Unit
OECD	The Organisation for Economic Co-operation and Development
OLS	Ordinary least squares
OOB	Out of bag error
PPCPs	Pharmaceuticals and Personal Care Products
RCP2.6	Representative concentration pathways 2.6
RCP8.5	Representative concentration pathways 8.5
RMSE	Root-mean-squared errors
RF	Random forest
TDS	Total dissolved solids
TU	Toxic Unit
WWTP	Wastewater treatment plant

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Chapter 1: Introduction and objectives

1.1. Point source and diffuse pollution in freshwater ecosystems

Freshwater resources can be adversely impacted by pollutants that originate from diffuse or point sources (Spanoghe et al., 2004). Point source pollution generally originates from the discharge at a discrete location such as pipes and ditches from the wastewater treatment plants, industrial effluents, confined intensive livestock operations, and combined sewer overflow (CSO, mixing urban runoff and sanitary system) (OECD, 2017). Point source pollution has a large impact on water quality in summer and dry periods due to low river and stream flows, as well as the reduction of dilution capacity, and during storm periods when combined sewer overflows operate more frequently (Even et al., 2007; Link et al., 2017; Mosley, 2015; Nilsson and Renöfält, 2008; Wang, 2014). Point sources of pollution are largely under control in OECD (Organisation for Economic Co-operation and Development) countries because they are easier to identify and more cost-effective to quantify, manage, and regulate (OECD, 2017).

In contrast to point source pollution, diffuse source pollution occurs when pollutants are discharged from agriculture to water resources through mechanisms such as spray drift, runoff, and leaching through the soil structure to groundwater during periods of rainfall and irrigation (Müller et al., 2002). Diffuse source pollution induces the most severe impacts in receiving water bodies during storm periods (particularly after a dry period) when rainfall induces hillslope hydrological processes and runoff containing pollutants from the land surface (OECD, 2017). Diffuse source pollution and its impacts on aquatic ecosystems and organisms largely remain challenges (e.g. decline in water quality, changes in chemical processes of major elements in water; alteration within species and community structure, and loss of biodiversity) due to their high spatio-temporal variability, making attribution of the pollution to sources complex. In addition, diffuse pollution control may require co-operation and agreement within catchments, and across sub-national jurisdictions and countries.

In recent years, the freshwater ecosystem has been facing serious threats from various types of pollutants (chemical, physical, radioactive, and pathogens) from both point and diffuse sources associated with human activities. Simultaneously, changes in abiotic factors such as precipitation and temperature in a warming climate may have altered the normal function of aquatic ecosystems and exacerbated the effects of pollutants on aquatic flora and fauna (Schmeller et al., 2018). Among the pollutants induced by human activities, pesticides represent an important stressor for freshwater ecosystems and can impact all groups of organisms (Beketov and Liess, 2008; Schäfer et al., 2011). Furthermore, anthropogenic activities have led to increasing salinisation that is reflected in a rise of electrical conductivity in freshwater ecosystems (Williams, 1987). Nevertheless, there is limited documentation on many pressing questions regarding pesticide and salinisation pollutions: to what extent wastewater treatment plants (WWTPs) contribute to freshwater pesticide pollution at large spatial scales; how baseline salinity might change in the future under climate change scenarios; how communities respond to increasing anthropogenic salinity; and whether freshwater taxa can adapt to background conditions. Therefore, studies that answer these questions are crucial to achieve a more sustainable management of freshwater ecosystems that protect aquatic life from negative impacts of anthropogenic activities. This thesis aims to tackle the four questions outlined above, comprising of two investigations of freshwater pollution: pesticide pollution originating from WWTPs (point source pollution), and salinity pollution induced by intrusions of salt into freshwater (diffuse source pollution) along with its effects on invertebrate turnover.

1.2. The spatial extent of freshwater pesticide pollution

Monitoring has revealed that pesticide residues regularly appear in surface water worldwide. Stone et al. (2014) found that 61 % of streams sampled had one or more pesticide compounds that were above chronic aquatic life benchmarks in agricultural catchments in the US during 2002-2011. In a review of Canada's national water quality surveillance program during 2003-2005, 90 % of surface water samples contained the herbicide 2-methyl-4-chlorophenoxyacetic acid (MCPA, Environment Canada, 2011), whereas MCPA was detected at 22.5, 43.2, 0.4, and 44.4 % of sites in Germany, France, the United States, and the Netherlands, respectively (Schreiner et al., 2016). Pesticide concentrations in streams also threaten freshwater biodiversity in the European Union (Malaj et al., 2014; Stehle and Schulz, 2015). A quarter (26 %) of all small agricultural German streams sampled contained one or more pesticides that exceeded the regulatory acceptable concentrations at least once during the 10-year

(2005 - 2015) monitoring period (Szöcs et al., 2017). Similarly, 45 % of all samples collected between 2002 and 2012 in the context of the Swedish environmental monitoring program contained one or more compounds that exceeded environmental quality targets of surface waters (Lindström et al., 2015). Results from a long-term, multi-catchment study of urban stormwater pesticides across Australia showed that pesticides including diuron, MCPA, 2,4-D, simazine, and triclopyr were found in more than 50 % of samples (Rippy et al., 2017). Monitoring programs across tropical countries such as Brazil, Chile, and Barbados, have detected several relatively polar pesticides in rainwater, surface runoff, and groundwater (Dores et al., 2008; Hill et al., 2016; Laabs et al., 2002; Lewis et al., 2016; Palma et al., 2004). Indeed, pesticides were responsible for the pollution of many freshwaters in Asian and African countries (Hasanuzzaman et al., 2018; Panuwet et al., 2012).

Several previous studies have either been carried out to represent pesticide pollution and its effects on surface water ecosystems and organisms, but were limited on temporal and spatial scales. For instance, many studies investigating pesticide pollution originating from WWTPs were limited to only a few WWTPs in the local or regional area and a small number of pesticide mixtures. Expanding on the previous investigations, one of the studies in this thesis focuses on assessing the contribution of WWTPs to pesticide toxicity move in small German agricultural streams on a large spatial scale from a large number of analyzed pesticides (Chapter 2). This allowed us to pinpoint the contribution of WWTPs to pesticide pollution in small agricultural catchments. We focused on small streams because pesticide toxicity in small streams has been previously reported to be generally higher compared to larger rivers (Lorenz et al., 2017; Schulz, 2004).

1.3. Freshwater pesticide pollution originating from wastewater treatment plants

The pesticide input into WWTPs mainly originates from associated agricultural or non-agricultural uses such as: improper waste disposal and accidental spillages; carelessness during filling and cleaning operations; urban uses such as grass management activities (e.g., golf courses, parks) and pest control in private homes and gardens, and biocidal uses in industry (Wittmer et al., 2010). Treated municipal wastewater largely meets current water quality standards for a wide range of substances including particulates, nutrients, and pathogens, but the removal of many micropollutants such as pesticides and pharmaceuticals is incomplete and highly variable (Eggen et al., 2014; Loos et al., 2013). Indeed, the removal efficiency for these substances depends largely on the treatment technique as well as the physicochemical properties of the compounds (Petrie et al., 2015; Tadkaew et al., 2011). For instance, the WWTPs effluent concentrations of sulfamethoxazole remain almost unchanged during activated sludge treatment. Consequently, sulfamethoxazole was frequently detected in effluents at raw concentrations of up to 81 % (Joss et al., 2005). Kasprzyk-Hordern et al. (2009) observed that notable concentrations of triclosan remained in the effluent, with a mean concentration of 25 μg L^{-1} (the triclosan concentration in WWTPs influent mostly ranges from 1.86 to 26.8 μg L^{-1}*)*.

Although pesticide concentrations in the effluent from WWTPs can generally be expected to be small due to extensive dilution (Neumann et al., 2002), elevated concentrations have been found downstream of such WWTPs (Campo et al., 2013; Gerecke et al., 2002; Munz et al., 2017), which then can affect receiving aquatic organisms (Ashauer, 2016; Bunzel et al., 2013). Several studies have emphasized the importance of WWTPs to pesticide toxicity in surface water. For example, Müller et al. (2002) observed that the pesticide residue was prevailed by WWTPs that contributed approximately 65 % of the total load entering the rivers in the Zwester Ohm, Germany. Similarly, up to 75 % of pesticides from urban uses entered two rivers (River Aabach and River Aa) through WWTPs in the catchment of Lake Greifensee, Switzerland (Gerecke et al., 2002). The WWTPs induced 65.9 % of the total herbicide load in two small streams: the Nette and its tributary the Pletschbach, Nordrhein-Westfalen (Neumman et al., 2002). The occurrence of pesticides from WWTPs regularly exceeds the ecotoxicological environmental quality standards (EQS) for individual compounds in surface water (Moschet et al., 2014; Wittmer et al., 2010).

1.4. Threats of pesticide pollution for freshwater species

Pesticides are applied massively in agricultural areas as they are chemicals engineered to reduce the prevalence of pest organisms. However, they can affect adjacent non-target ecosystems such as rivers and streams (Schulz, 2004). Consequently, the application of pesticides may involve aquatic quality degradation, irreparable biodiversity loss, and deterioration of ecosystem services (European Commission, 2015). Recently, a review ranked pesticides as the second most important driver for the worldwide decline in insect populations (Sánchez-Bayo and Wyckhuys, 2019). Neonicotinoid insecticides, pyrethroid insectides, and fipronil can have a devastating impact on aquatic insects and crustaceans due to their high acute and chronic toxicity (Beketov and Liess, 2008; Kasai et al., 2016; Roessink et al., 2013), thus reducing their abundance in freshwater (Dijk et al., 2013). Pesticides caused loss of up to 42 % taxa in streams in Europe (Germany and France) and Australia (southern Victoria) (Beketov et al., 2013).

Studies have linked pesticides to freshwater pollution (Stehle and Schulz, 2015), and to adverse effects on fish survival (Weston et al., 2014), ecosystem functions (Schäfer et al., 2011, 2007), and biodiversity (Geiger et al., 2010; Weston et al., 2014). Additionally, in aquatic ecosystems, pesticides typically co-occur forming mixtures (Altenburger et al., 2015; Malaj et al., 2014; Schäfer et al., 2013; Schreiner et al., 2016), which vary in composition and concentration over time and space due to the usage of different pesticides on various crops in the same basin area (Oerke, 2006), the application of multiple pesticides on a single crop to combat the increasing resistance of pests (Whalon et al., 2008), and mixtures of various active ingredients in a single pesticide product (Altenburger et al., 2013; Relyea, 2009). Extensive research has shown that the impacts of pesticide mixtures on aquatic species can strongly exceed the impacts of single compounds (Brack et al., 2015; Gustavsson et al., 2017; Rodney et al., 2013).

1.5. Salt ions, causes, and extent of freshwater salinisation

Besides the input of pesticides, salinity in rivers has been perceived as another major pollutant in several countries (Cañedo-Argüelles et al., 2013). The major cations and anions making up salinity in freshwater are sodium (Na^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^+), chloride (Cl^-), sulphate (SO_4^{2-}), and carbonate (CO_3^{2-}) (Tanji and Kielen, 2002). Electricity conductivity (EC) in miliSiemens per centimeter (mS cm^{-1}) and total dissolved solids (TDS) in grams per liter (g L^{-1} TDS ~ 1.65 mS cm^{-1}) are frequently used for the total salinity parameter. Although chloride concentrations have received most of the scientific and public attention as a dominant form of salt pollution (Kaushal et al., 2013), other salt ions, and EC can also contribute to salinisation. The composition of salt ions varied greatly in various freshwaters due to the influence of natural and anthropogenic factors (Cormier and Suter, 2013).

Freshwater salinisation is caused by natural factors (primary salinisation) and anthropogenic activities (secondary salinisation). Without the presence of anthropogenic influences, salinity and the proportions of salt ions originate from several natural sources including rock weathering, sea intrusion, rainfall, and atmospheric deposition (Comte and Banton, 2006; Herczeg et al., 2001; Neilson-Welch and Smith, 2001; Williams, 1987). Secondary salinisation, considered as a proxy for human-driven salinisation, has risen the salinity levels above historical levels in many freshwater ecosystems around the world. Kaushal et al. (2018) estimated that salinisation has impacted 37 % of the drainage area of the contiguous United States (US). Among the different causes of freshwater salinisation, road de-icing seems to be the major source of salts (mainly NaCl) in the USA (Chapra et al., 2012; Corsi et al., 2015; Godwin et al., 2003, 2003; Interlandi and Crockett, 2003; Kaushal et al., 2005; Kelly et al., 2008; Novotny et al., 2009; Rosfjord et al., 2007). Nevertheless, salt in freshwater also originates from many other activities such as construction, agriculture, and mining (Bernhardt and Palmer, 2011; Fritz et al., 2010; Johnson et al., 2010; Kaushal et al., 2013, 2008; Moquet et al., 2014; Palmer et al., 2010; Raymond et al., 2008; Steele and Aitkenhead-Peterson, 2011). Freshwater salinisation will expand and increase in the future in the US. Olson, (2019) predicted the median EC to increase from 0.319 mS cm^{-1} to 0.524 mS cm^{-1} with over 50 % of streams having greater than 50 % increases in EC and 35 % of streams more than doubling their EC by 2100. Many of Australia's freshwater ecosystems have been degraded by increasing salinity as a result of rising saline groundwater due to the clearing of natural deep-rooted native vegetation from catchments, passive flow into rivers (Kefford et al., 2006; Muschal, 2006; Williams, 2001) and modifications to the water. It is estimated that up to 41,300 km of streams and lakes in Australia could be at risk from shallow water tables or have a high salinity hazard by the year 2050 (The National Land and Water Resource Audit, 2001). Salinity concentrations in Australian rivers and wetlands were expected to increase from less than 500 mg L^{-1} to over 10,000 mg L^{-1} in the next 50 years (Nielsen et al., 2003).

Saline freshwaters are not restricted to the US and Australia, European countries such as Spain and Germany are also facing salinisation due to potash and coal mining. Estévez et al. (2019) showed that around 27 % of the streams in Spain were saline (SR > 0 where SR is the salinisation ratio of conductivity predicted by the model and maximum absolute conductivity measured in good status). Furthermore, the EC measured in the Llobregat basin (northeast Spain) was 3 – 4 times higher than that of seawater due to discharge from a potash mine. During the period of intense mining activities from 1950s to 1980s, the peak of salinity in streams in the Llobregat basin reached EC levels of up to 132.4 mS cm^{-1} (Ladrera et al., 2017). In Germany, peak chloride concentrations in the Werra and Lippe rivers reached more than 30 g L^{-1} and 3.5 g L^{-1}, due to a long history of being affected by brines from coal mining areas (Coring and Bäthe, 2011; Petruck and Stöffler, 2011). Salinisation of Asian rivers and streams has been associated with urban development (Bhatt and McDowell, 2007) and chemical, paper, and textile industries (Lokhande et al., 2011; Nirgude and Shukla, 2013). Increase in the salinity of North American and African freshwater originated from salt factories, drainage canals, salt extraction, and agriculture (Achem et al., 2015; Akomolafe and Onwusiri, 2017; Bazzuri et al., 2018; Lokhande et al., 2011; Scherman et al., 2003).

Although increasing freshwater salinisation has been investigated in many previous studies, there is a lack of estimation of how pervasive it might be, or how much background levels may change in the context of climate change. One of the objectives in this thesis was to set empirical models background salinity using major drivers of freshwater salinisation involving geological, climatic, and environmental factors. Such models predict the alterations and the range of change that will possibly occur under future climate change scenarios (Chapter 3).

1.6. Salinisation effects on freshwater species

Salinisation of freshwater has been responsible for a significant decline in biodiversity (Halse et al., 2003; Piscart et al., 2005) and ecosystem functions (Berger et al., 2019). Although the response may differ among organisms and communities, salinisation usually leads to a reduction in species richness primarily through its link with osmoregulatory physiology. The freshwater organisms must expend energy to sustain a sufficient internal osmotic pressure associated with their surrounding environment. As salt levels in the external environment exceed certain levels, it requires a rising osmoregulatory attempt associated with a relative energy requirement, which may lead to cell damage and death of individuals and finally whole populations (Cañedo-Argüelles et al., 2019; Kefford et al., 2016). Salinity was responsible for the disappearance of several taxa in many freshwaters, e.g Meurthe River, Northeastern France (Piscart et al., 2005), a wetland in Western Australia

(Pinder et al., 2005), and the lowland river Lippe, Western Germany (Schröder et al., 2015). Salinisation also affects taxa richness by shifting from salt-sensitive to salt-tolerant taxa adapted to increased salinity concentrations (Braukmann and Böhme, 2011; Dunlop et al., 2005; Horrigan et al., 2005). Piscart et al. (2005) observed that a salinity range between 0.8 and 1.0 mS cm^{-1} caused the shifts from saline-sensitive taxa to communities with more tolerant taxa. Other than increased osmotic stress and loss of salt-sensitive taxa, increasing salinity induced shifts in the food web resulting in a different composition of invertebrate functional feeding groups (Kefford et al., 2012). A study conducted in 20 saline prairie lakes across southern Saskatchewan, Canada found that zooplankton occurred over a very large salinity gradient, but the taxonomic composition changed as salinity increased. Subsequently, the complexity of the fish community (diversity) was associated with large changes in invertebrate communities (Cooper and Wissel, 2012).

Although many previous studies have demonstrated the relationship between increased absolute salinity and the change/loss in freshwater species richness, we lack studies that highlight the general aspect of species turnover based on salinity change due to human-induced salinisation. Therefore, in contrast to previous studies, we investigated the response of invertebrate richness in terms of species turnover (as the number of species eliminated and replaced per unit time, and focus on equilibrium state in terms of immigration and extinction in a population (MacArthur and Wilson, 1967)) to levels of salinity change (EC change in our case, chapter 4) rather than absolute EC. Our investigation represents a new approach to determining the impacts of anthropogenic salinisation on freshwater species communities and may contribute to identifying the development of adaptations.

1.7. Aims and research questions of the thesis

This thesis investigated 1) point source pollution, exemplarily by the case of pesticides originating from WWTPs in small agricultural streams on a large spatial scale, and 2) diffuse pollution in the case of salt ions in freshwater associated with changes in invertebrate communities. The specific research questions are:

1. Do WWTPs contribute to pesticide toxicity in small agricultural streams on a large spatial scale? (Chapter 2)

2. Is pesticide toxicity associated with distance from upstream WWTPs? (Chapter 2)

3. What are the major drivers of freshwater background salinisation? (Chapter 3)

4. How may future background salinity change under scenarios of future climate change? (Chapter 3)

5. How do invertebrate communities in terms of species turnover respond to levels of EC change, considered as a proxy for human-driven salinisation? (Chapter 4)

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Chapter 2: Contribution of waste water treatment plants to pesticide toxicity in agriculture catchments

Ecotoxicology and Environmental Safety, 145 (2017) 135-141

2.1. Abstract

Pesticide residues are frequently found in water bodies and may threaten freshwater ecosystems and biodiversity. In addition to runoff or leaching from treated agricultural fields, pesticides may enter streams via effluents from wastewater treatment plants (WWTPs). We compared the pesticide toxicity in terms of log maximum Toxic Unit (log mTU) of sampling sites in small agricultural streams of Germany with and without WWTPs in the upstream catchments. We found an approximately half log unit higher pesticide toxicity for sampling sites with WWTPs ($p < 0.001$). Compared to fungicides and insecticides, herbicides contributed most to the total pesticide toxicity in streams with WWTPs. A few compounds (diuron, terbuthylazin, isoproturon, terbutryn and Metazachlor) dominated the herbicide toxicity. Pesticide toxicity was not correlated with upstream distance to WWTP (Spearman's rank correlation, $rho = -0.11$, $p > 0.05$) suggesting that other context variables are more important to explain WWTP-driven pesticide toxicity. Our results suggest that WWTPs contribute to pesticide toxicity in German streams.

2.2. Introduction

Pesticide residues in surface water are among the major stressors for freshwater ecosystems (Malaj et al., 2014). Depending on compound specific properties such as persistence in water, stability to degradation, bio-accumulation potential and toxicity, pesticides can affect non-target organisms such as microorganism, invertebrates, plants and fish (Köhler and Triebskorn, 2013). This is particularly important for small water bodies, which contribute a disproportionally high proportion of total freshwater biodiversity (Schulz, 2004; Biggs et al., 2014). However, compared to larger water bodies, small water bodies receive considerably higher inputs of micropollutants due to their higher interconnectedness with the surrounding landscape and lower dilution potential (Lorenz et al., 2016; Munz et al., 2017; Neale et al., 2017; Szöcs et al., 2017). Hence, the predicted ecological effects are often stronger than in larger streams or rivers (Lorenz et al., 2016).

Pesticides enter water bodies via diffuse input paths including subsurface leaching and surface runoff, where the amount transported to surface waters depends on many factors, including soil properties, topography, climate (temperature, rainfall, moisture and wind), crop type and pesticide properties (Leonard, 1990; Kreuger, 1998; Ramos et al., 2000). Wastewater treatment plants represent a major point source for pesticide entry (Gerecke et al., 2002; Campo et al., 2013). Pesticide pollution of wastewater can be attributed to careless disposal of pesticide-containers and equipment washing and urban use such as gardening, vector control and biocidal use, e.g., in paints (Wittmer et al., 2010). Given incomplete degradation of some compounds (Maldonado et al., 2006), pesticides present in wastewater can pass through WWTPs and discharging directly into surface water.

Several studies have examined the pesticide pollution originating from WWTPs. However, many previous studies were limited (1) in scale (a small number of WWTPs on the local or regional scale) and (2) to a few pesticide mixtures. For instance, a study of three WWTPs in Catalonia, Northeast Spain found total concentrations of 22 selected pesticide compounds to be below 1 µg L^{-1} in effluents in most instances and the removal ratio of these compounds in WWTPs was variable and often very low (Köck-Schulmeyer et al., 2013). Levels of five fungicides (8.82 – 73.39 ng L^{-1}) entering the Acheloos river (Etoloakarnania, Greece) from WWTPs were found in a study of Stamatis et al. (2010). Other studies were carried out on a regional level, but as well limited to a few compounds or WWTPs (Gerecke et al., 2002; Neumann et al., 2002; Berenzen et al., 2003; Kahle et al., 2008). Finally, a small fraction of studies on pesticide toxicity from WWTPs was conducted in small water bodies, and most of these were limited to a few sites (Lorenz et al., 2016). Thus, to date a large scale study on the pesticide pollution of small streams from WWTPs is lacking.

We aimed at assessing the contribution of WWTPs to pesticide toxicity in small agricultural streams on a large spatial scale using pesticide monitoring data from Germany. Given that diffuse pesticide inputs into agricultural streams is widespread, we hypothesized that in such catchments WWTPs play a minor role. The monitoring data comprised a large number of analyzed pesticides (312 compounds). We quantified the difference in pesticide toxicity between sampling sites with and without WWTPs in the upstream catchments. In addition, we assessed whether pesticide toxicity was related to the upstream distance to WWTPs.

2.3. Methods

2.3.1. Data sets and catchment selection

Pesticide concentrations in streams, measured from 2005 to 2015, and the location of sampling sites were provided by the German federal state authorities and the Working Group on Water of the German federal states (LAWA). Pesticide monitoring data was available for eleven of the 16 German states (Fig 2.1).

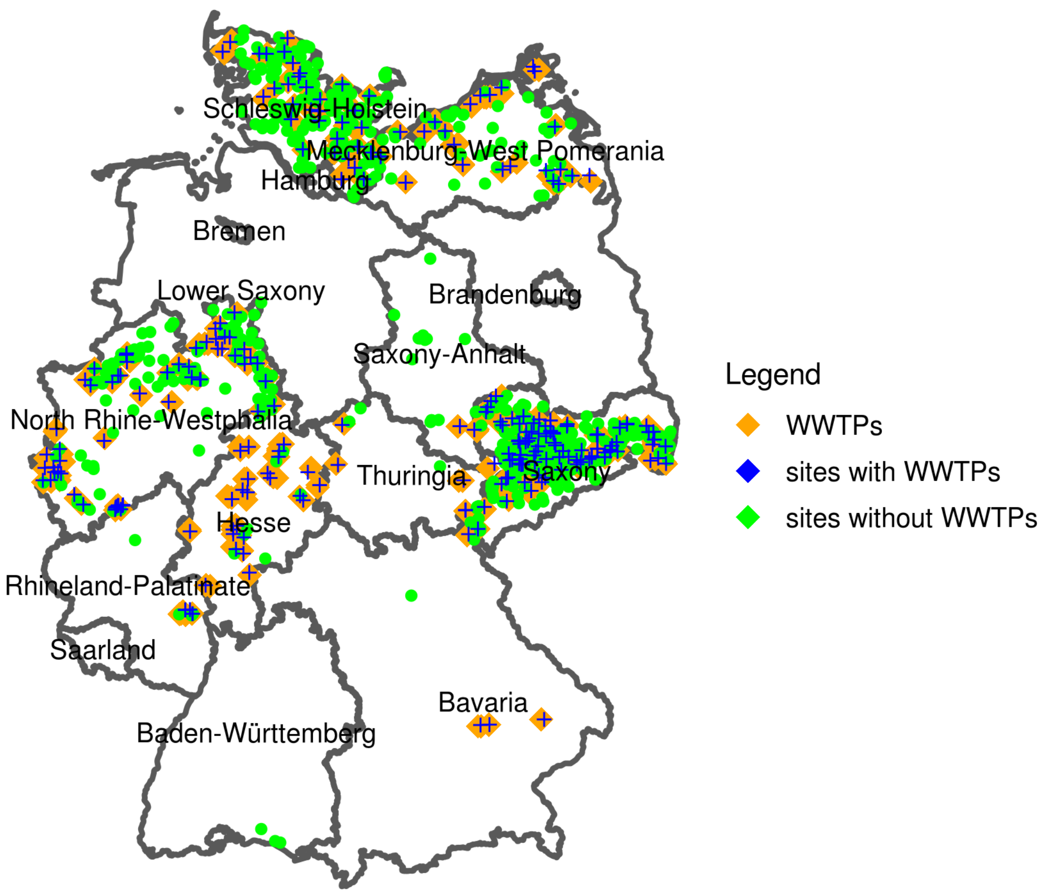


Fig 2.1: Location of samplings with and without WWTPs

The pesticide samples consisted of grab water samples that were retrieved from sampling sites in small streams in largely fixed intervals. The total number of analyzed pesticides across all sites was 312 including 145 herbicides, 95 insecticides and 72 fungicides and was approximately equal across the groups of sites (Fig 2.2). Similarly, the average number of pesticides analyzed per site exhibited no significant difference (all p > 0.08, t-test) between sites with and without WWTPs (Fig 2.3).

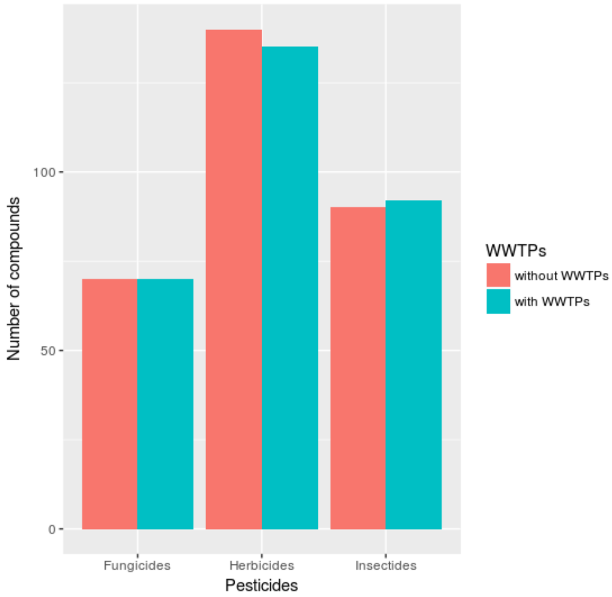
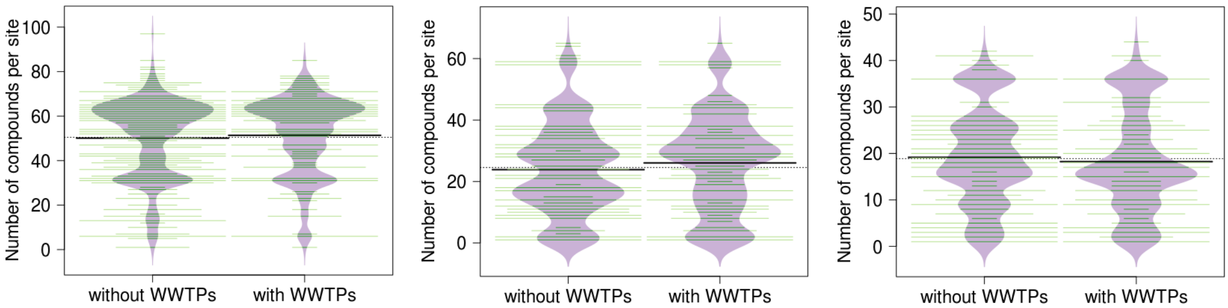


Fig 2.2: Total number of herbicides, insecticides and fungicides analyzed in sites with and without WWTPs



a. b. c.

Fig 2.3: Plot of number of compounds per site with and without WWTPs for herbicides (a); insecticides (b); and fungicides (c). None of the groups differed significantly (Student's t-test p = 0.36 (herbicides), p = 0.08 (insecticides) and p = 0.28 (fungicides).Green lines give individual data points, with the length of the line indicating the relative frequency, the thick black line gives the mean value per group, the dashed black line gives the mean value over both groups.

Regarding spatial data, geographical information on the WWTPs and agricultural land use were extracted from the Authoritative Topographic-Cartographic Information System (ATKIS) for Germany (Adv, 2016). For each sampling site, upstream catchments were derived from a digital elevation model (DEM) (EEA, 2013) based on the multiple flow direction algorithm (Holmgren, 1994) as implemented in GRASS GIS 7 (Metz et al., 2011; Neteler et al., 2012). In some flat areas, the algorithm failed to delineate catchments (16 % of all sites). In this case, the catchments were assigned based on information of drainage basins provided by the Federal Institute of Hydrology (BfG) for the respective stream segments, and the derived information was amalgamated with the other data.

The contribution of WWTPs to pesticide pollution in agricultural catchments was evaluated in this study. We also expected that due to the widespread diffuse pollution their contribution is of minor relevance. In addition, our analysis was limited to small agricultural catchments, as pesticide toxicity in small streams has been previously reported to be generally higher compared to larger rivers (Schulz,2004; Lorenz et al., 2016). We selected 812 sampling sites according to these two criteria: (1) catchment area below 30 km^2 and (2) more than 40 % agricultural land use in the catchment. Catchments to 40 % agriculture were used because previous studies showed turning points for ecological change and pesticide concentrations near this threshold (Feld, 2013; Waite, 2014; Szöcs et al., 2017).

2.3.2. Sampling site grouping

Sampling sites were grouped according to the presence/absence of WWTPs in the upstream catchment. For those catchments that were derived based on stream segment catchments (i.e., where the outlet of the catchment did not match exactly with the sampling site), we confirmed manually whether the WWTPs were upstream of the sampling site. A total of 244 sampling sites had at least one WWTP in the upstream catchment, whereas 568 sites had no WWTP.

2.3.3. Calculation of Toxic units

Pesticide acute toxic units were defined as the ratio of the measured pesticide concentration and the toxicity of this compound measured as the median acute lethal concentration (LC_{50}) for a specific species (species j).

$$\mathrm{TU}=\frac{C_i}{\mathrm{LC}\,50_{\mathrm{ij}}} \quad \text{(Eq2. 1)}$$

where TU_i is the acute toxic unit of an individual pesticide compound i; C_i is the measured concentration [μg L^{-1}] of the individual pesticide compound in water. Concentrations below the quantification limit were excluded from this analysis. In case of insecticides and fungicides, arthropods usually are the most sensitive aquatic species (Maltby et al., 2005), thus *Daphnia magna* was used as the reference species (48-h acute toxicity test). In addition, data on other arthropod species is limited (Schäfer et al., 2013). In case of neonicotinoid insecticides *D. magna* was shown unsuitable for assessing general acute and chronic toxicity (Jemec et al., 2007; Beketov and Liess, 2008; Ashauer et al., 2011). Therefore, we used data of other most sensitive arthropods (Appendix A1 Table A1.1, EC_{50}, 48 – 96 h). In case of herbicides, vascular plants and algae usually comprise the most sensitive groups (Brink et al., 2006). Therefore, we used the green algae *Pseudokirchneriella subcapitata* as the reference species (EC_{50}, 48 – 96 h). Toxicity data were extracted from Schäfer et al. (2011) Schüürmann et al. (2011), Malaj et al. (2014) and ECOTOX US. EPA (EPA, 2016). Missing values (mainly for green algae) were estimated using Ecosar v1.11 (EPA, 2012) and Chemprop (UFZ, 2016).

We used the maximum Toxic Unit (mTU), which is defined as the highest TU_i of all detected substances in each site over the whole sampling period, as an indicator for the minimal expected toxicity at the respective site (Schäfer et al., 2013). The metric has been successfully applied to evaluate effects of pesticides on stream macroinvertebrates in multiple studies (Schäfer et al., 2012).

2.3.4. Statistics

The Student t-test was used to compare sites with and without WWTPs for total pesticide toxicity and individually for different pesticide groups: herbicides, insecticides and fungicides. The mTUs were logarithm-transformed (log mTU) to yield normal distribution of the data. The non-parametric correlation coecient rho was used to assess the correlation of the mTU with the distance from each sampling site to the closest upstream WWTP for the 244 sites with WWTPs. All calculations and graphics were performed in R version 3.3.1 (R Core Team, 2016).

2.4. Results and discussion

2.4.1. Pesticide toxicity at sites with and without WWTPs

The average log mTU for sites with WWTPs was approximately a half log unit higher than for sites without WWTPs (Fig 2.4; − 1.46 and − 1.92 for sites with and without WWTPs, respectively, t-test $p < 0.001$). The difference was not due to differences in the number of compounds analyzed between sites with and without WWTPs (Fig 2.2 and 2.3). Similarly, Munz et al. (2017) found higher pesticides concentrations downstream from WWTPs during low flow conditions with high inputs of dimethoate, pirimicarb and thiacloprid. A recent study of Neale et al. (2017) also found that wastewater eluents were relevant sources of micropollutants including pesticides in small water bodies.

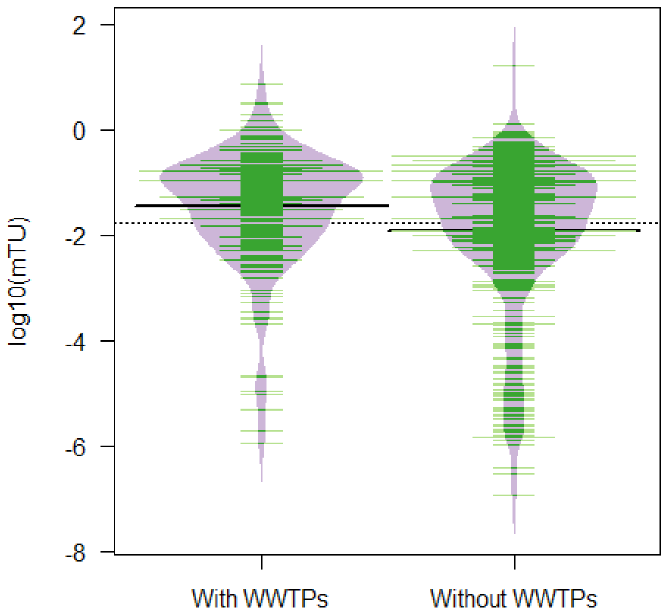


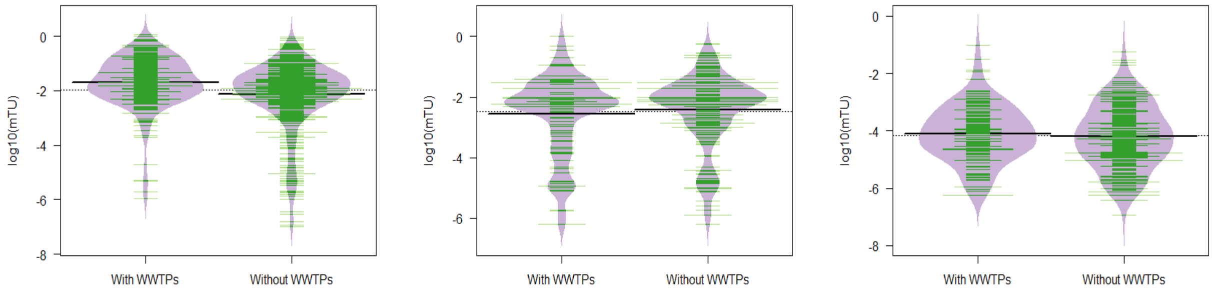
Fig 2.4: Plot of log(mTU) for sampling sites with and without WWTPs in the upstream catchment. Green lines give individual data points, with the length of the line indicating the relative frequency, the thick black line gives the mean value per group, the dashed black line gives the mean value over both groups.

With respect to different pesticide groups, the highest average toxicity was found for herbicides (Fig 2.5). In addition, herbicides exhibited a similar difference between sites with and without WWTPs of approximately half a log unit (− 1.7 and − 2.1, respectively, Student's t-test $p<0.001$) as all pesticide groups combined. By contrast, the log mTU values for sites with and without WWTPs for fungicides and insecticides did not differ significantly (t-test $p = 0.39$ and $p = 0.37$, respectively). Hence, in most sites herbicides influenced the total mTU and were demonstrated in this study to contribute to pesticide toxicity from WWTPs. Moreover, the mTU of each group was influenced by a few individual pesticides such as diuron, terbuthylazin, chlorpyrifos, p,p-DDT, tebuconazole and boscalid (Table 2.1).

Compared to herbicides, insecticides and fungicides are characterized by lower concentrations in surface waters due to generally higher hydrophobicity, lower mass applied and shorter application periods (Neumann et al., 2002; Stone et al., 2014; Moschet et al., 2014). In addition, grab water sampling is likely to underestimate hydrophobic chemicals in small streams that predominantly enter streams via runoff and require precipitation event-driven sampling for appropriate characterisation (Stehle et al., 2013; Szöcs et al., 2017). Thus, our study most likely underestimated the general occurrence of pesticides in streams and particularly of hydrophobic insecticides and fungicides, which means that the relative contribution of pesticide groups to the toxicity should be interpreted with this caution. Finally, more herbicides were measured in total and per sample and this may contribute to higher toxicity for herbicides, though the number of measured compounds would need to be compared to the applied number herbicides, insecticides and fungicides.

Due to the selection criteria for the sampling sites (small catchments < 30 km^2 and > 40 % agricultural land use in the catchment) and due to the fact that we relied on monitoring data carried out by the respective federal authorities the sampling sites were spatially unevenly distributed and mainly concentrated in three areas of Germany (see Fig 2.1). The uneven spatial distribution raises the question to which extent the results are representative for the whole of Germany. For example, if agricultural practice, treatment effeciency, or WWTP size would vary between states, inferring from a spatially biased sample to the whole of Germany could lead to over- or underestimation of the contribution of WWTPs to toxicity. This could be resolved through more detailed information on the WWTPs (e.g., population equivalents, treatment efficiency), which was unavailable for this study. Nevertheless, this does not affect our main conclusion that WWTP are a relevant entry path for pesticide-driven toxicity.

This study shows that WWTPs are a potential source of pesticides in small agricultural streams. This suggests incomplete degradation during wastewater treatment. The treatment processes in WWTPs are designed to control a wide range of substances including particulates, nutrients and pathogens. While the treatment efficiency is generally high for these substances, the performance of pesticide removal can be poor and is highly variable (Eggen et al., 2014). Many pesticides are only partially degraded during the treatment process, which has been reported in several studies (Morasch et al., 2010; Singer et al., 2010; Stamatis et al., 2010; Bueno et al., 2012; Campo et al., 2013; Köck-Schulmeyer et al.,2013). Depending on the compound, removal effeciencies in a few randomly selected studies on European WWTPs ranged between 0 % and 72 %, except for Clotrimazole where removal efficiency was between 85 % and 94 % (Appendix A1, Table A1.2).



a. b. c.

Fig 2.5: Plot of log(mTU) from sampling sites with and without WWTPs for herbicides (a); insecticides (b); and fungicides (c). The log mTU did not differ significantly for insecticides (p=0.39) and fungicides (p=0.37). Herbicides exhibited a significant difference of approximately half a log unit in the mTU(− 1.7and − 2.1,respectively, Student's t-test p<0.001). Green lines give individual data points, with the length of the line indicating the relative frequency, the thick black line gives the mean value per group, the dashed black line gives the mean value over both groups

Table 2.1: List of ten most important pesticide compounds for each pesticide group with the number of cases where the compound accounted for the highest mTU and log mTU range for sites with and without WWTPs

Compounds	Sites with WWTPs		Sites without WWTPs	
	No. cases compound accountable for max mTU	Range of log mTU with WWTPs	No. cases compound accountable for max mTU	Range of log mTU without WWTPs
Herbicides				
Diuron	72	-2.3 to 0.1	82	-2.6 to -0.2
Terbuthylazin	36	-3.1 to 0.0	97	-3.1 to -0.5
Isoproturon	35	-3.7 to -0.2	89	-3.2 to 0.0
Terbutryn	26	-2.5 to -0.7	32	-2.6 to -0.3
Metazachlor	23	-3.2 to -0.7	66	-3.1 to -0.3
Chlortoluron	9	-2.5 to -0.4	13	-2.6 to -0.8
Bentazon	2	-5.3 to -4.7	11	-5.5 to -2.5
Dimethachlor	2	-2.8 to -1.4	3	-3.0 to -2.4
Metolachlor	2	-3.1 to -2.7	13	-3.6 to -1.1
Pendimethalin	2	-2.3 to -2.2	8	-3.0 to -1.2
Glyphosate	0	-	6	-6.0 to -5.3
Insecticides				
Chlorpyrifos	54	-2.3 to 0.0	106	-2.3 to -0.3
p,p-DDT	17	-3.2 to -0.7	42	-3.2 to -0.6
4,6-Dinitro-o-Cresol	13	-5.1 to -3.7	19	-5.1 to -3.4
Imidacloprid	10	-2.7 to -2.2	0	-
Dimethoat	5	-5.0 to -3.0	5	-4.9 to -3.5

Pirimicarb	6	-3.5 to -1.4	14	-3.5 to -0.6
Clothianidin	4	-3.6 to -3.2	10	-3.6 to -2.1
HCH, gamma (Lindan)	4	-6.2 to -5.7	7	-6.2 to -5.1
Thiacloprid	3	-2.1 to -1.6	15	-2.5 to -1.2
Dicofol	3	-3.8 to -2.7	0	-
Dichlorvos	0	-	2	-1.3 to -0.2
o,p-DDT	1	0.05	5	-2.9 to -1.8
Fungicides				
Tebuconazol	34	-5.0 to -3.0	56	-5.0 to -3.0
Boscalid	21	-6.2 to -3.5	43	-6.4 to -4.7
Carbendazim	19	-4.4 to -2.6	42	-4.8 to -2.4
Azoxystrobin	17	-4.9 to -3.1	27	-4.9 to -2.3
Dimoxystrobin	15	-4.3 to -1.0	34	-4.3 to -2.4
Hexachlorbenzen	13	-4.8 to -2.7	30	-4.8 to -1.6
Cyprodinil	7	-3.8 to -2.2	7	-3.7 to -2.6
Propiconazol	7	-6.1 to -3.4	23	-6.2 to -3.2
Fenpropimorph	6	-5.7 to -3.5	6	-6.2 to -3.1
Metalaxyl	2	-5.5	3	-5.9 to -5.3
Difenoconazol	1	-2.0	7	-2.6 to -1.2

In addition, hydrolysis and desorption during treatment processes in WWTPs could contribute to the elevated pesticide concentrations at sites with WWTPs. This can be explained by the presence of some substances in the influent of WWTPs, e.g. metabolites and transformation products, which can subsequently be back-transformed to pesticide compounds during biological wastewater treatment (Singeretal.,2010;

Köck-Schulmeyer et al., 2013; Campo et al., 2013). A negative elimination rate for mecoprop was found with the levels of this pesticide in the tertiary effluent 11 % higher than in the primary effluent (Singer etal., 2010). The authors suggested that mecoprop leached into WWTPs as an ester (e.g. ethylhexylester, octylester, polyethylenglykol) with hydrolysis resulting in mecoprop during the treatment processes. To sum up, the incomplete removal during wastewater treatment as well as potential transformation processes are likely the reason for the elevated pesticide toxicity in streams with WWTPs.

The total average of pesticide toxicity in terms of log mTU was −1.8, where 31 % of sites had a log mTU > −1 (Fig 2.4). Mesocosm studies found considerable effects on aquatic communities for log TU values exceeding −1 (Brock et al., 2000; Van Wijngaarden et al., 2005). A meta-analysis of field studies suggested up to 42 % of loss of stream invertebrate species at such levels of pesticide toxicity (Beketov et al., 2013). Species loss is particularly important in small streams because they have a higher beta diversity, i.e. host higher proportions of biodiversity (Davies et al., 2007; Biggs et al., 2014). The findings of Neale et al. (2017) and Munz et al. (2017) related to the pesticide input from WWTPs into small streams in Switzerland confirm the ecological risks from pesticides.

To reduce the discharge of micropollutants such as pesticides from WWTPs, advanced wastewater treatments have been developed such as ozonation and advanced oxidation processes (Hollender et al., 2009). A study found that six micropollutants were considerably removed (> 79 %) at ozone concentrations of 15 mg L^{-1} in the biological treatment (Hernández-Leal et al., 2011). Likewise, Gerrity et al. (2011) applied O_3/H_2O_2 for removing micropollutants (PPCPs, pesticides and steroid hormones) during water reclamation and reported considerable removal efficiency (> 90 %) for most of compounds. Moreover, 48 out of 52 micropollutants (PPCPs and pesticides) in WWTP effluent were removed to below their limit of detection using photo-Fenton with solar light (Klamerth et al., 2010). Finally, studies found an increase of the number of sensitive taxa and increase ecosystem functioning in terms of leaf decomposition during ozonation compared to the pre-ozonation period (Bundschuh et al., 2011; Ashauer, 2016). Thus, advanced wastewater treatment may contribute to a reduction in pesticide input from WWTPs and an improvement in the ecological situation.

2.4.2. Relationship between toxicity and distance to WWTP

The distances to upstream WWTPs were not associated with maximum pesticide toxicity (mTU) of 244 sampling sites with WWTPs (Spearman correlation coefficient rho = − 0.11, p = 0.09). This confirms that the distance may not be relevant to predict pesticide toxicity from WWTPs. We expected that a larger distance would result in a lower pesticide toxicity due to instream processes (i.e., dilution, degradation, sorption). The lack of such a relationship with distance might be attributed to the variable dilution potentials of the streams (Link et al., 2017). Additionally, some other variables such as physical mixing of upstream water and WWTP effluents, fraction of wastewater effluent downstream, potential daily variations in effluent discharge, flow conditions, abiotic factors including temperature and nutrients in surface water bodies may also be stronger determinants of WWTP driven pesticide toxicity (Reiss et al., 2002; Neale et al., 2017).

2.5. Conclusions

WWTPs contribute to pesticide toxicity in small agricultural streams because of incomplete removal of pesticides as well as metabolites and transformation products that are potentially transformed during wastewater treatment processes. Compared to insecticides and fungicides, herbicides contributed the most of total pesticide pollution from WWTPs in this study due to generally lower hydrophobicity, higher mass applied and longer application periods. The lack of relationship between the non-transformed toxicity data (mTU) and distance to WWTP might be attributed to the variable dilution potentials of the streams, however, we suggest that it is worth mentioning negative results.

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Chapter 3: Predicting current and future background ion concentrations in German surface water under climate change

Phil. Trans. R. Soc B 374: 20180004.

3.1. Abstract

Salinisation of surface waters is a global environmental issue that can pose a regional risk to freshwater organisms, potentially leading to high environmental and economic costs. Global environmental change including climate and land use change can increase the transport of ions into surface waters. We fit both multiple linear regression (LR) and random forest (RF) models on a large spatial dataset to predict Ca^{2+} (266 sites), Mg^{2+} (266 sites), and SO_4^{2-} (357 sites) ion concentrations as well as electrical conductivity (EC - a proxy for total dissolved solids with 410 sites) in German running water bodies. Predictions in both types of models were driven by the major factors controlling salinity including geologic and soil properties, climate, vegetation and topography. The predictive power of the two types of models was very similar, with RF explaining 71 – 76 % of the spatial variation in ion concentrations and LR explaining 70 – 75 % of the variance. Mean squared errors for predictions were all smaller than 0.06. The factors most strongly associated with stream ion concentrations varied among models but rock chemistry and climate were the most dominant. The RF model was subsequently used to forecast the changes in EC that were likely to occur for the period of 2070 to 2100 in response to just climate change—i.e. no additional effects of other anthropogenic activities. The future forecasting shows approximately 10 % and 15 % increases in mean EC for representative concentration pathways 2.6 and 8.5 (RCP2.6 and RCP8.5) scenarios, respectively.

3.2. Introduction

Two types of processes can be distinguished that govern an increase of salinity: primary and secondary salinisation. Primary salinisation is associated with increasing salt input originating from natural processes such as rainfall, rock weathering, sea-water intrusion and aerosol deposits (Williams, 1987). Human-driven salinisation is called secondary salinisation and is mainly induced by land development, agriculture, discharge of industrial liquid or solid waste, mining, road de-icing or intensive fertilization, and irrigation (Cañedo-Argüelles et al., 2013; Williams et al., 2000; Ziemann et al., 2001). The effects of stream salinisation on water bodies range from physiological responses of organisms (Kefford et al., 2011) to alterations in freshwater communities, to subsequent reductions in ecosystem functioning (Schäfer et al., 2012). Reductions in the density and species richness of multiple organism groups such as diatoms (Busse et al., 1999), macroinvertebrates (Cañedo-Argüelles et al., 2012; Kefford et al., 2011), amphibians (Odum, 1988) fish (and riparian plant communities (Lymbery et al., 2003; Vandersande et al., 2001) have been reported in response to increasing surface water salinity.

Climate change can accelerate natural salinisation processes, thereby increasing the salt load to water bodies. For example, modelling of climatic forcing of the water and chloride balance for freshwater lake Ijsselmeer in the Netherlands shows that the peak chloride concentrations can increase by up to 108 mg l^{-1} (an increase of 14.3 % compared to the reference situation) through the climate change scenario W+ (characterized by a strong increase in the global mean temperature) in 2050. The main driver for the increase is the changing hydrology of the Rhine River expected to change owing to snowmelt and rainfall that dominate river water volume (Bonte and Zwolsman, 2010); the case studies in six lakes and reservoirs in southern Europe (Estonia, Greece, Turkey and Italy) and the Middle East (Israel), and one in Brazil reveal that changes in water level owing to climate warming have significant effects on salinity level and trophic structure of lakes and reservoirs (Jeppesen et al., 2015). Future climate change projections for Central and eastern Europe forecast an increase in temperature extremes, such as an increase in the duration and intensity of droughts (Anders et al., 2014). This may exacerbate salinisation in this region. Although salinisation in Central European countries such as Germany is currently considered rather a localized problem, mostly originating from mining (Szöcs et al., 2014), other studies indicated that salinisation can interfere with other stressors. For example, increasing salinity was a major factor controlling the invasion of alien species (molluscs and crustaceans) in German streams (Früh et al., 2012). Finally, other human activities may interact with and exacerbate the natural processes of salinisation. Natural salts in water bodies could be enhanced through human-accelerated weathering (Kaushal et al., 2018, 2017, 2013). For example, the disturbance of lithology through urban construction can bring bedrock materials to the surface that are subject to chemical weathering (Siver et al., 1996).

Natural background ion concentrations are driven by salt input originating from natural processes such as rainfall, rock weathering, sea-water intrusion and aerosol deposits without the presence of human activities. Models predicting baseline salinity help to establish benchmark conditions that can be used to assess whether stream water quality has degraded through secondary salinisation. Moreover, such models inform on the changes and the range of variation that are likely to occur compared to baseline salinity under different scenarios of future climate change. Thereby, they also represent a first step towards investigating future salinity, including human drivers. Generally, such information is required to assess and communicate the economic costs of ongoing and future river salinisation and thus to make decisions for management regarding mitigation and local adaptation.

The major factors that control the natural background level of salinity are lithology, climate, vegetation, relief and soil properties (Nédeltcheva et al., 2006; Olson and Hawkins, 2012; Reimann et al., 2009; Riebe et al., 2003; Rothwell et al., 2010; Stallard and Edmond, 1983), hydrochemical processes, size and elevation of the watersheds (Olson and Hawkins, 2012; Pratt and Chang, 2012; Rothwell et al., 2010) and groundwater (Last, 1992; Woocay and Walton, 2008). Here, we modelled and forecast the change in natural background ion concentrations in running waters of Germany based on these major factors, focusing on electrical conductivity (EC, a proxy for total dissolved solids), Ca^{2+}, Mg^{2+} and SO_4^{2-}. Major drivers of salinisation (temperature and with its resulting evaporation, dilution, etc.) are likely to change. Hence, our study evaluates the importance of the different drivers and gives a first indication of likely consequences of climate change on salinisation.

3.3. Material and methods

3.3.1. General approach

We used the major natural factors controlling salinity such as geologic and soil properties, climate, vegetation and topography as predictors in models. We then compared the predictive power of random forest (RF) and LR models for EC. We used RF models because they can model nonlinear relationships, are insensitive to over fitting and generally have high predictive performance compared with other machine-learning methods (Breiman, 2001; Hastie et al., 2009; Liaw and Wiener., 2002). However, since machine-learning approaches are often complex to interpret, we also fitted a statistical model. Since the ordinary linear regression (LR) model can perform poorly in both prediction and interpretability of results in situations with intercorrelated predictors (Zou and Hastie, 2005), we used a penalized approach, i.e. the elastic net to overcome these drawbacks. Assessment of model performance, caveats and limitations of models are presented in subsequent sections. The model with the best predictive performance was used to forecast EC in German streams for the period from 2070 to 2100.

3.3.2. Datasets and catchment selection

Data for EC and ion concentrations in streams, measured from 2005 to 2015, and the location of sampling sites were provided by the German federal state authorities (Fig 3.1 and Appendix A2, Fig A2.1). To identify sites that represent background EC and ion concentrations without major human influence, we selected sites according to the following two criteria: (1) less than 5 % agricultural/urban land use in the catchments, (2) no mining in the catchments. These criteria of site selection were based on Olson & Hawkins (2012), Herlihy *et al*., (2008) and Herlihy & Sifneos., (2008).

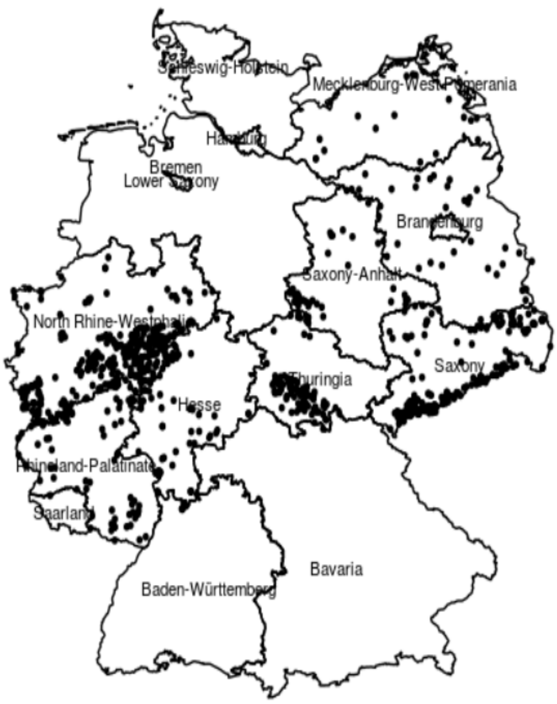

Fig 3.1: Location of EC monitoring sites (other ions see Appendix A2, Fig A2.1)

The source of geologic data used in the study is The Geological Survey Map of the Federal Republic of Germany 1:200000 (GÜK200) (see https://www.bgr.bund.de/EN/Themen/SammlungenGrundlagen/GG_geol_Info/Karten/Deutschland/GUEK200/guek200_inhalt_en.html, accessed on 16 July 2018). The map consists of 55 layers that give the distribution of more than 3800 geological units of the surface geology of Germany and adjacent areas. The geological units contain information on the stratigraphy (age), genesis and component lithologies of the rocks. The spatially dominant lithology was estimated for each geological unit based on 102 different lithologies listed by the GÜK200. Then, we characterized five attributes for each unit comprising percentages of CaO, MgO and S; uniaxial compressive strength as a proxy for rock strength and hydraulic conductivity as a proxy for rock–water interaction. The values of the five attributes for each lithology were derived from Olson & Hawkins., (2012) (Appendix A2,Table A2.1).

Climatic data such as mean annual temperature, precipitation and the number of days of freeze were obtained from the DWD Climate Data Center in Germany (see https://www.dwd.de/EN/climate_environment/cdc/cdc_node.html, accessed 16 July 2018). Multi-annual grids of precipitation, air temperature (2 m above ground) and freeze days over Germany for 1981 - 2015 at a resolution of 1 km × 1 km were used. For further variables related to soil properties, vegetation and groundwater recharge velocity, Appendix A2, Table A2.2.

Geographical information on mining, agricultural, conservation and urban land use was extracted from the Authoritative Topographic-Cartographic Information System for Germany (ATKIS)(Adv). For each sampling site, upstream catchments were derived from a digital elevation model (Rothwell et al., 2010) based on the multiple flow direction algorithm (Holmgren, 1994) as implemented in GRASS GIS 7 (Metz et al., 2011; Neteler et al., 2012). During the derivation of upstream catchments, we also calculated topographic indices such as the area and elevation for each catchment. In some flat areas, the algorithm failed to delineate catchments (16 % of all sites). In these cases, the catchments were assigned based on information of drainage basins provided by the Federal Institute of Hydrology for the respective stream segments, and the derived information was amalgamated with the other data (WasserBLIcK, 2015).

3.3.3. Modeling

There was no evidence of spatial autocorrelation, as indicated by semivariograms calculated using the R packages SSN (Hoef and Peterson, 2018) and openSTARS (Kattwinkel and Szöcs, 2018) (Appendix A2 Fig A2.2); therefore, no adjustments for spatial autocorrelation were needed. LR and RF models were used to develop predictive models of natural background EC and ion concentrations in streams in Germany. We compared the predictive power of both models. We considered four responses (EC, Ca^{2+}, Mg^{2+} and SO_4^{2-}) and used 19 candidate predictive variables: 5 describing geological characteristics, 3 describing climate, 7 describing soil properties, 2 capturing topography, 1 each for vegetation and groundwater (Table 3.1).

Table 3.1: Response and predictor variables. Time periods for the mean variables are described in Appendix A2, Table A2.2.

Response/ predictor variable	Category of variable	Variable	Unit
Response	EC	Electrical conductivity (EC)	mS cm^{-1}
	Ion concentration	Ca^{2+}	mg l^{-1}
		Mg^{2+}	mg l^{-1}
		SO_4^{2-}	mg l^{-1}
Predictor	Geology	Catchment mean CaO	%
		Catchment mean MgO	%
		Catchment mean S	%
		Catchment mean unconfined compressive strength	MPa
		Catchment mean log geometric mean hydraulic conductivity	10^{-6} m s^{-1}
	Climate	Catchment mean annual temperature	°C
		Catchment mean annual precipitation	mm
		Catchment mean number of freeze days	days
	Soil	Catchment mean available water capacity	Dimensionless

Response/ predictor variable	Category of variable	Variable	Unit
		Catchment mean bulk density	g cm^{-3}
		Catchment mean organic matter content	%
		Catchment mean soil erodibility	Dimensionless
		Catchment mean soil permeability (k_f)	m s^{-1}
		Catchment mean soil depth	m
		Catchment mean water table depth	m
	Topography	Catchment area	km^2
		Catchment mean elevation	m
	Vegetation	Catchment mean enhanced vegetation index (EVI)	Dimensionless
	Groundwater	Catchment mean recharge speed	mm a^{-1}

Linear regression model

The LR model is given by: $y = \beta_0 + \beta_1 \times_1 + \beta_2 \times_2 + \ldots + \beta_p x_p$, where $x_1, \ldots, x_p$ are predictors; y is the response and p is number of predictors. The vector of regression coefficients β (β_0, β_2, … β_p) is derived in model fitting, for example, using ordinary least squares (OLS) by minimizing the residual sum of squares. However, OLS can perform poorly in both prediction and interpretability of results, particularly with intercorrelated predictors (Zou and Hastie, 2005). Penalized approaches such as the LASSO, ridge regression and the elastic net have been suggested to improve OLS. The LASSO imposes an L_1-penalty on the regression coefficients and simultaneously does both continuous shrinkage and automatic variable selection such that only the important predictor variables remain in the model. On the other hand, by bounding on L_2-norm of the coefficients and continuous shrinkage, ridge regression (Hoerl and Kennard, 1970) can minimize root-mean-squared errors (RMSE) and achieve higher prediction performance. The regression coefficients in these techniques are shrunk towards zero by imposing a penalty on their size (Hastie et al., 2009). Bühlmann & van de Geer (Bühlmann and Geer, 2011) showed that when predictor variables are correlated, analyses with the elastic net can result in a lower mean squared error (MSE) than LASSO and ridge regression. Moreover, the use of the elastic net method has been shown to identify a higher number of influential variables than LASSO and ridge regression approaches (Tutz and Ulbricht, 2009).

Given that many predictors were highly correlated (Appendix A2, Table A2.3), we fitted LR models by applying the elastic net (R package glmnet) (Friedman et al., 2018). The elastic net represents a combination of ridge regression and LASSO as suggested by Zou & Hastie (Zou and Hastie, 2005) employs the elastic net penalty $P(\beta)$ composed of two component penalty functions:

$$P(\beta) = \sum_{j=1}^{p} \left(\frac{1}{2}(1-\alpha)\beta_j^2 + \alpha|\beta_j| \right) \quad \text{(Eq3. 1)}$$

The first penalty is the ridge penalty (L_2) that minimizes the weighted sum of squared regression coefficients, whereas the second component is the LASSO penalty (L_1) minimizing the weighted sum of absolute regression coefficients. The penalty parameter $\alpha \in (0,1)$ determines the bias variance trade-off between L_1 and L_2 (i.e. how much weight should be given to either the LASSO or ridge regression). The elastic net with $\alpha = 0$ performs ridge regression, whereas $\alpha = 1$ is equivalent to the LASSO; β denotes the values of the regression coefficients.

We tuned α and λ in our models and selected the optimal model as the α and λ combination (Appendix A2, Table A2.4) that yielded the highest prediction performance based on five-fold cross-validation. The λ is the shrinkage parameter selected from a range of 0.0001 to 1 and hyper-parameter α ranged from 0 to 1. Before developing the LR models, we applied spread-level plots (car package; Fox, 2018) to assess the residuals for heteroscedasticity which suggested that the response variable be log-transformed. We also log-transformed the predictor variables catchment area and organic matter content to improve linearity between these predictors and the response variables. Predictor variables with a larger coefficient are considered to be most important.

Random forests

RF is a machine-learning method introduced by Breiman (Breiman, 2001) to enhance predictive accuracy and classification accuracy without overfitting data. RF is based on the principle of classification and regression trees (CART). RF uses several bootstrap samples of the data that are randomly selected at each node as a subset of explanatory variables to build many binary decision trees (Breiman, 2001). We used the R package randomForest for fitting RF (Liaw and Wiener, 2002).

We checked for variables that exhibit very low variance and removed highly correlated variables that were unlikely to be informative. We also used partial dependence plots to identify predictors with uninterpretable relationships with the responses, and we removed these predictors from the final models. By implementing the function tuneRF with 1500 trees, the optimal number of terminal nodes (mtry) at each node that produced the minimum out of bag error (OOB error) was determined (Appendix A2, Table A2.5). In detail, a bootstrap sample of the original data was used to construct each tree in the tuneRF function. The observations that are not used to construct a tree are denoted out-of-bag (OOB) observations. OOB observations can then be predicted from the trees to evaluate prediction accuracy, where the resulting error is referred to as OOB error. The best performing model (optimal mtry) is identified as the one with minimum OOB error. Variable importance was evaluated as the mean decrease in accuracy (% IncMSE), a measure of how much the model error increases if that variable's information is removed by randomizing it.

3.3.4. Assessment of model performance

In elastic net regression, we applied a five-fold cross-validation approach to estimate the model with the highest predictive accuracy, whereas in RF the OOB error was used to identify the best model. To compare the models, we also calculated the coefficient of determination (R^2) and the MSE of the variance for both models. The R^2 is a goodness of fit measure, whereas MSE is an absolute measure of predictive accuracy. All calculations and graphics were performed in R version 3.3.1 (R Core Team, 2018).

3.3.5. Forecasting future EC

We examined the effects of likely climate change (temperature and precipitation) on future EC, while holding all other factors constant (i.e. geology, soil properties, vegetation and groundwater). We selected 610 standard water monitoring sampling sites in small German streams that are spatially evenly distributed and therefore representative for the whole of Germany (UBA - German Federal Environmental Protection Agency). The dataset of environmental factors for these 610 sites was extracted from the same sources as described in Table 3.1, however, with the future data for temperature and precipitation. We applied the established RF model that showed the best predictive performance to the new dataset to predict current and forecast future EC. Then, we calculated EC alteration as the difference between the predicted 2070–2100 EC (both scenarios RCP2.6 and RCP8.5) and the baseline scenario to identify any tendencies of natural salinity to change in Germany's streams in the future.

For climate change projections, we used statistically downscaled datasets from the Delta Method for the Fifth Assessment Report of Intergovernmental Panel on Climate Change (ICPP, 2013). The CSIRO MK. 3.6.0 model was applied with a 30 s resolution for the time period 2070–2100 under the RCP 2.6 and RCP 8.5 (RCP—representative concentration pathways) for future climate change. While the RCP 2.6 represents one of the scenarios that aims to limit the increase of global mean temperature to 2°C, RCP 8.5 is based on a comparatively high greenhouse gas emissions scenario where the range of temperature increase is expected to be 3.5 – 4.5°C by 2100 (ICPP, 2013).

3.4. Result and discussion

3.4.1. Model fit, model evaluation and important variable

RF generally resulted in more parsimonious models compared with LR. The number of selected predictors in the models ranged from 12 for Mg^{2+} to 19 for both Ca^{2+} and SO_4^{2-} in the LR models, and from 7 for Ca^{2+} to 10 for SO_4^{2-} in the RF models (Appendix A2, Table A2.6). We found clear differences among ions in the relative importance of the factors for predicting stream chemistry. Nevertheless, the most dominant factors in predictive models were rock chemistry and climate (Appendix A2, Table A2.6). Previously, Nédeltcheva et al. (2006) found annual rainfall and the proportion of different minerals in the bedrock to be the two main factors driving stream water chemistry, Olson & Hawkins, (2012); Olson, (2019) showed that both rock chemistry and climate were important predictors of stream chemistry. Stream chemical composition in Queensland is a consequence of both geological history, and past and present climates (McNeil et al., 2005).

Stream Ca^{2+}, Mg^{2+} and SO_4^{2-} concentrations were positively correlated with percentages of CaO, MgO and S in the catchment rock, representing the effect of different bed rock types on stream chemistry, caused primarily by the geochemical weathering and erosion. During chemical weathering, these elements become separated from the rocks as dissolved ions or colloids. Subsequently, they may be incorporated into secondary minerals or remain as primary resisted minerals. Solutions that have reacted with rocks and contain dissolved ions, colloids and suspended matter will eventually reach surface water bodies and thus increase concentrations of Ca^{2+}, Mg^{2+} and SO_4^{2-} that make up a large portion of EC. Based on catchment studies in the USA, Walling (Walling, 1980) found that ion concentrations in surface water were independent of solubilities of the minerals present in these rocks. Since ultrabasic rocks are rich with pyroxenes and olivine, the predominant ion expected from these rocks in surface water is Mg^{2+}. Similarly, Ca^{2+} is the dominant cationic contribution in water bodies from calcareous or dolomitic soils.

EC and ion concentrations in streams correlated negatively with mean annual precipitation. This result likely indicates that increasing precipitation results in large water volumes in streams, which causes dilution of most solutes (Stallard and Edmond, 1983; Williams, 2001). Moreover, the amount of precipitation also influences the amount of flow through the soil as well as the soil water retention time before it enters streams and lakes. We found a positive relation between temperature and EC, and between temperature and ion concentrations, which may also indicate an effect of water volume. An increase in water temperature increases evaporation (Sereda et al., 2011), which in turn reduces dilution capacity, translating into higher salinity (Crowther and Hynes, 1977).

Between 70 % and 75 % of the variance in stream water EC, Ca^{2+}, Mg^{2+} and SO_4^{2-} was explained by environmental factors in LR (Fig 3.2 and Appendix A2, Fig A2.3), respectively, with MSEs all less than 0.06. The predictive power of RF and LR models were likely similar, with RF explaining 71 – 76 % of the spatial variation in ion concentrations and LR explaining 70 - 75 % of the variance (Fig 3.2 and Appendix A2, Fig A2.4). The SO_4^{2-} concentration was best explained in both models (R^2 = 76 %, MSE = 0.033 in RF; R^2 = 75 %, MSE = 0.043 % in LR).

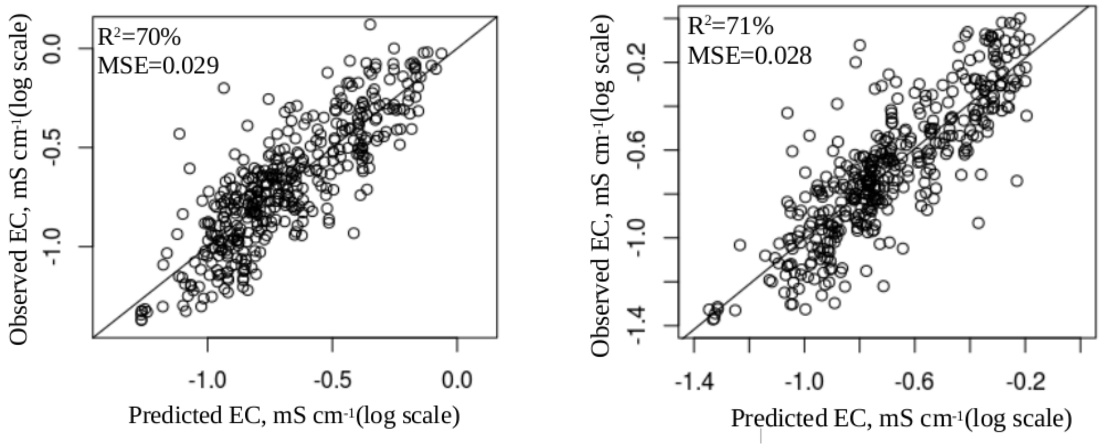

Fig 3.2: Plots of predicted versus observed EC value in a) LR b) RF (other ions see Appendix A2, Fig A2.3 and Fig A2.4).

3.4.2. Forecast future EC

For Germany, climate change is expected to result in increased mean temperature and a decrease in mean precipitation until end of the century, based on the selected scenarios (Appendix A2, Fig A2.5 and Table A2.7). Evaporation is expected to rise when temperature increases (Sereda et al., 2011). A decrease in the amount of precipitation results in a decrease in catchment runoff (Nielsen and Brock, 2009). Reduced river discharge implies a lower dilution capacity that also contributes to higher salinity concentration (Crowther and Hynes, 1977).

We forecast an approximately 10 – 15 % increase in mean EC for the climate change scenarios for German small streams. The average ECs in the period from 2070 to 2100 for scenarios RCP 2.6 and RCP 8.5 were predicted to be 0.407 (± 0.008 (= 2 s.e.)) and 0.418 (± 0.007) mS cm^{-1} compared with 0.366 (± 0.010) mS cm^{-1} for the period from 2005 to 2015 (Fig 3.3). Furthermore, the magnitude of the difference between the two scenarios in forecast EC was not as large as expected (0.407 versus 0.418 mS cm^{-1}) because precipitation was considered the most important factor in stream chemistry in the RF model, but there was only a slight difference in predicted precipitation between the two scenarios (Appendix A2, Fig A2.5b).

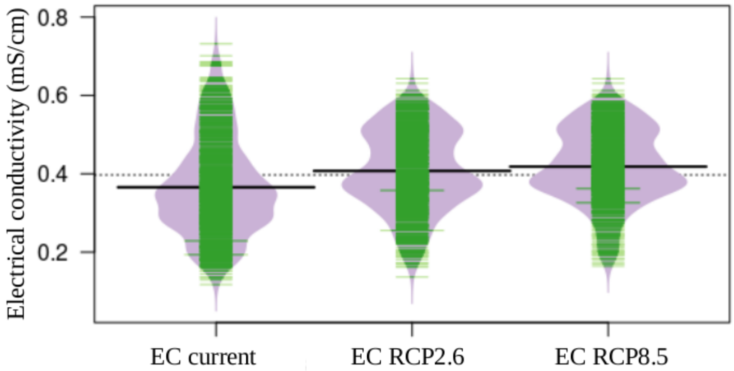

Fig 3.3: Plot of EC current values (2005-2015), forecast values (2070-2100) in RCP 2.6 and RCP 8.5 scenario. Green lines give individual data points, with the length of the line indicating the relative frequency, the thick black line gives the mean value per group, the dashed black line gives the mean value over all groups.

An increase in EC was also found over the past decades in the United States (US) and Australia but mainly driven by anthropogenic factors including mining, resources extraction, agriculture and urbanization. A large proportion of the streams (37 %) in the US have been impacted by increasing EC over the past 50 years (Kaushal et al., 2018) and the predicted rate of increase in EC is 50 % (Olson, 2019). In Australia, the predicted rates of average river salinity change for the period from 1998 to 2100 showed strong regional differences. The average of salinity at the lower River Murray (Southeast Australia) was predicted to increase from 0.57 to 0.90 mS cm^{-1} in 100 years (i.e. 58 %), whereas this rate was 60 % and 505 % for Central East and Northeast Australia, respectively (The Muray Darling Basin Commisson, 1999, 2015).

Statistically increasing trends in EC were observed at approximately 80 % of sites (610 sites, Appendix A2, Fig A2.6) for RCP2.6 and RCP8.5, whereas this proportion is 37 % for the US (Kaushal et al., 2018). This finding suggests a more homogeneous response in Germany, which is not surprising given the wider gradients in climate and lithology in the US. In some cases, the predicted change in EC was substantial: for example, EC in RCP2.6 and RCP 8.5 was predicted to increase by 50 % at 5 % and 10 % of sites, respectively, mostly in south Germany (Fig 3.4). An increased EC can negatively impact ecosystem processes (Bonte and Zwolsman, 2010; Crowther and Hynes, 1977). Populations of invertebrates (e.g *Heterocypris* sp.) may be reduced from present levels if streams salinity increases by more than 50 % (Galat et al., 1988). Shifting food habits and reducing fish production are likely consequences of a salinity-induced disruption in the benthic invertebrate forage base (Galat et al., 1988). A large-scale study by Kefford et al. (2011) found approximately 13 %, 9 %, 12 %, 8 %, 4 % and 20 % species loss in all taxa, all insects, EPT, non-EPT insects, crustaceans and molluscs, respectively, for a 30 % change in EC in Southeast Australia, leading to community changes (Kefford et al., 2010). Finally, loss in community trait diversity was linear along the salinity gradient (Schäfer et al., 2011)

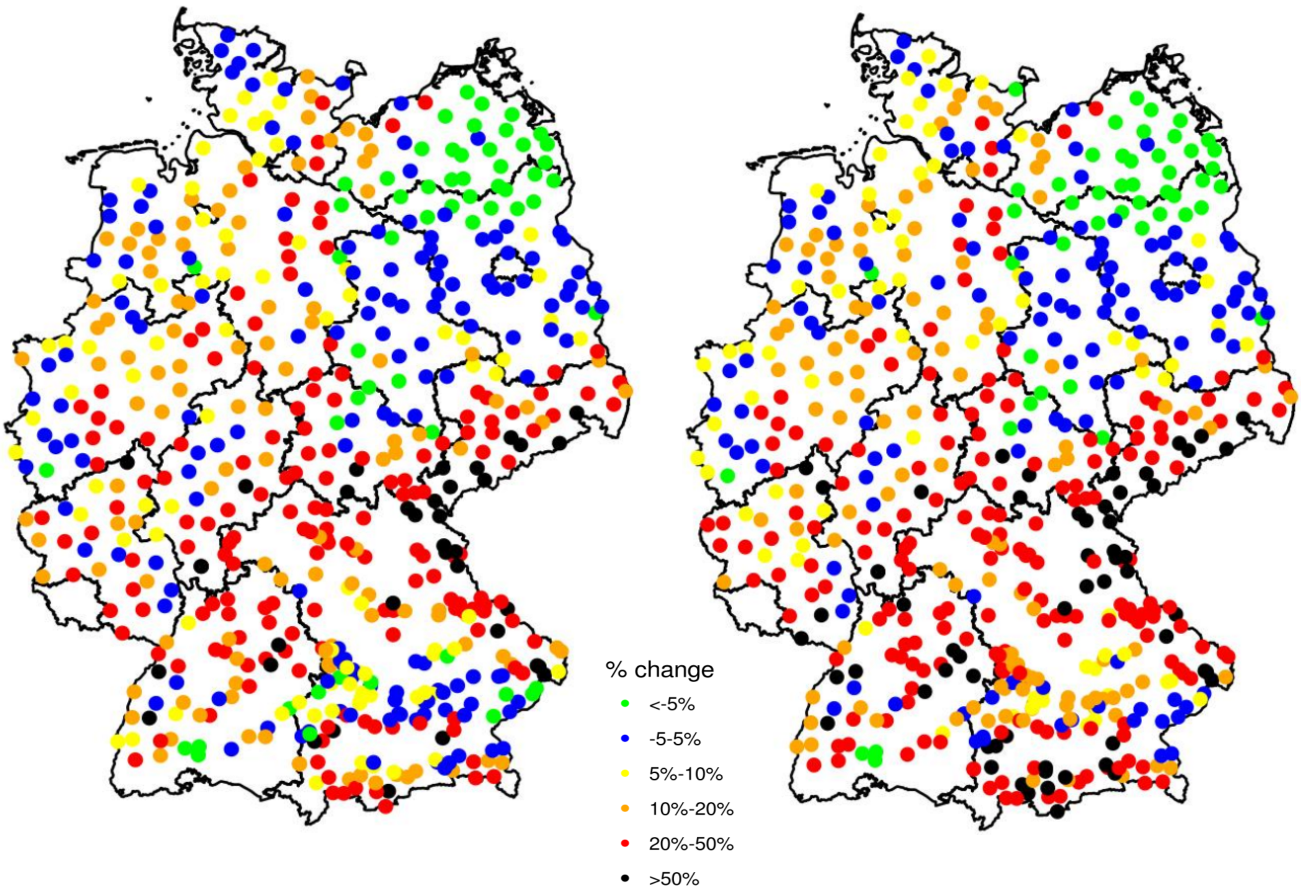

Fig 3.4: Percent change in scenario a) RCP 2.6 b) RCP 8.5

3.4.3. Model limitations

First, owing to the selection criteria for the sampling sites (less than 5 % agricultural and urban land use in the catchments, no mining) and owing to the fact that we relied on data monitored by the respective federal authorities, the sampling sites were spatially unevenly distributed and some areas of Germany lacked sampling sites (Fig 3.1 and Appendix A2, Fig A2.1). Therefore, some environments were not adequately represented in our models (especially environments in Bavaria with high elevation and more continental climates) and our predications for these areas may be biased. Second, natural salinisation processes can interact with other human and non-human processes. For example, increasing oceanic alkalinity has been driven by an interplay of acidic precipitation, amongst other factors (Müller et al., 2016). Acid rain interacts with lithology through weathering to increase dissolved inorganic carbon in river water and in turn conductivity (Kaushal et al., 2013). Such factors (e.g. pH of precipitation or atmospheric deposition (Ca^{2+}, Mg^{2+}, SO_4^{2-} and other minerals)) were omitted from our model, which means that our forecast may underestimate the increase in natural background salinity. Finally, extrapolating statistical relationships outside the range of observed data should be interpreted with care. In other words, if the new predictor values are outside of the range on which the model was fit, the relationship between the independent variables and the dependent variable might change outside of that range (Conn et al., 2015). The extreme temperatures expected in the future exceed the range of measured temperature (Appendix A2, Fig A2.5) in our study, which likely causes RF to underestimate the actual change in salinity at these sites. Most climate models estimate future temperatures higher than current ones; therefore, this issue is likely to be a general one in statistical models.

3.5. Conclusion

We fit two statistical models (LR and RF) to forecast ion concentrations and EC in streams in Germany. The model findings may on the one hand directly inform on potential risks in other Central European regions with similar gradients in lithology and climate. On the other hand, the models may be adopted in other European regions with similar major drivers in stream chemistry to begin to establish a continent-wide assessment of both current and future changes in salinity. The results of EC projections show a slightly elevated conductivity in German streams in the period from 2070 to 2100 under climate change. Changes in other human and non-human processes such as changes in the acidity of atmospheric deposition or land use may exacerbate natural salinisation processes, though incorporation of such processes was beyond the scope of this study. In particular, incorporating land use information may enhance our predictive capacity and understanding of the future anthropogenic influence on stream salinity.

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Chapter 4: Invertebrate turnover along gradients of anthropogenic salinisation in rivers of two German regions

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4.1. Abstract

Rising salinity in freshwater ecosystems can affect community composition. Previous studies mainly focused on changes in freshwater communities along gradients of absolute levels of electrical conductivity (EC). However, both geogenic and anthropogenic drivers contribute to the EC level and taxa may regionally be adapted to geogenic EC levels. Therefore, we examined the turnover in freshwater invertebrates along gradients of anthropogenic EC change in two regions of Germany. The anthropogenic change of EC was estimated as the difference between the measured EC and the modeled background EC driven by geochemical and climate variables. Turnover in freshwater invertebrates (β-diversity) was estimated using the Jaccard index (JI). We found that invertebrate turnover between EC gradient categories is generally greater than 47 %, with a maximum of approximately 70 % in sites with a more than 0.4 mS cm^{-1} change compared to the baseline (i.e. no difference between predicted and measured EC). The invertebrates *Amphinemura sp.*, *Anomalopterygella chauviniana* and *Leuctra sp*. were reliable indicators of low EC change, whereas *Potamopyrgus antipodarum* indicated sites with the highest EC change. Variability within categories of EC change was slightly lower than within categories of absolute EC. Elevated nutrient concentrations that are often linked to land use may have contributed to the observed change of the invertebrate richness and can exacerbate effects of EC on communities in water. Overall, our study suggests that the change in EC, quantified as the difference between measured EC and modeled background EC, can be used to examine the response of invertebrate communities to increasing anthropogenic salinity concentrations in rivers. However, due to the strong correlation between EC change and observed EC in our study regions, the response to these two variables were very similar. Further studies in areas where EC change and observed EC are less correlated are required. In addition, such studies should consider the change in specific ions.

4.2. Introduction

Salinity refers to the total concentration of dissolved inorganic ions in water (Williams and Sherwood, 1994) and is usually measured as the water capacity to conduct electrical current (electrical conductivity, EC). The salinity levels in water vary widely and are related to natural processes (rainfall, rock weathering, sea-water intrusion and aerosol deposits) (Williams, 1987) and anthropogenic activities within the catchment such as land development, agriculture, discharge of industrial liquid or solid waste, mining, road de-icing or intensive fertilization, and irrigation (Cañedo-Argüelles et al., 2013; Kaushal et al., 2018; Williams et al., 2000; Ziemann et al., 2001). In Germany, the potash industry is the major anthropogenic source of salts in rivers with the dominant ions being chloride, potassium, sodium, magnesium, and sulphate (Bäthe and Coring, 2011; Braukmann and Böhme, 2011; Petruck and Stöffler, 2011; Schulz and Cañedo-Argüelles, 2019; Ziemann and Schulz, 2011). However, especially since the 1990ies, the discharge of salt from mining has declined and in turn lead to a reduction in mining-related salinity levels in German rivers. For example, the chloride concentrations in the Lippe River, North Rhine-Westphalia, were up to 3500 mg L^{-1} (~ EC = 9.5 mS cm^{-1}) in the first part of last century, whereas the mean concentration is now below 400 mg L^{-1} (~ EC = 3.2 mS cm^{-1}, Petruck and Stöffler, 2011). During the period of intense mining activities from 1950s to 1980s, the chloride concentrations in the Werra river, Thuringia, reached up to 30 g L^{-1} (~ EC = 65.5 mS cm^{-1}), which was higher than the salinity levels of the North Sea (Bäthe, 1997). However, since 2000 the chloride concentration decreased to 2500 mg L^{-1} (~ EC = 7.5 mS cm^{-1}) at Gerstungen and to less than 400 mg L^{-1} (~ EC = 3.2 mS cm^{-1}) at Bremen, located 505 km downstream (Bäthe and Coring, 2011). This reduction and the smoothened amplitudes of the chloride concentrations have resulted in a gradual recovery of the aquatic fauna (Szöcs et al., 2014).

Recent studies identified geological and climatic drivers as the main natural drivers of EC in Central European and Northern American surface waters (Le et al., 2019; Olson and Hawkins, 2012). These studies allow estimating the EC in a site determined by natural processes (hereafter called background EC) without anthropogenic influence. Hence, the difference between the estimated background EC and the observed EC in a site (hereafter EC change) can serve as a proxy of human influence, which typically increases the EC.

Rising salinity in water bodies can affect aquatic organisms in multiple ways, including direct toxic effects as well as changed chemical processes in streams. Freshwater invertebrates are highly sensitive to elevated levels of salts (Pinder et al., 2005; Wolf et al., 2009) and their communities can be significantly altered by rising salinity (Braukmann and Böhme, 2011; Hart et al., 1991; Kefford et al., 2011). Studies in Australian water bodies predicted that increasing salinisation will reduce the biodiversity of macroinvertebrates. Pinder et al., (2005) suggested that up to 100 species are at risk of extinction with the expansion of salinisation in Southwest Australia, representing one of the regions strongest affected by salinisation (NLWRA, 2001). However, loss of biodiversity with increasing salinity has also been observed in Central Europe. The species richness decreased along the salinity gradient in the Meurthe River, Northeastern France, with a total loss of 30 % of the taxa as salinity exceeded 1400 mg L^{-1} (Piscart et al., 2005). The discharge of salt-enriched mining in Western Germany has impacted macroinvertebrate and diatom communities, with major changes in both communities at an EC exceeding 0.9 mS cm^{-1} (Schröder et al., 2015). Conversely, long-term studies in a central German river found an increase in macroinvertebrate diversity after freshwater management lead to a reduction in mining-related salinity levels (Bäthe and Coring, 2011; Szöcs et al., 2014).

Organisms can adapt to their environment through phenotypic plasticity as well as through genetics and epigenetics. Short- and long-term adaptations in response to EC levels were found in many studies for a wide range of organisms (e.g. sticklebacks (DeFaveri and Merilä, 2014; McCairns and Bernatchez, 2010), Atlantic killifish (Velotta et al., 2014; Whitehead et al., 2011), amphibians (Hopkins and Brodie, 2015), and the invertebrates *Eurytemora affinis* (Posavi et al., 2014), *Daphnia pulex* (Weider and Hebert, 1987), and *Daphnia magna* (Jeremias et al., 2018)). Hence, within limits, invertebrates may locally be adapted to the background EC. Most previous studies examined the response of invertebrate communities to observed levels of EC, determined by the background EC and potential human influences. However, given potential adaptations, the communities may relate stronger to EC change than to the observed EC, in particular where the observed EC is mainly driven by the background EC. Therefore, in contrast to previous studies, we examined the response of invertebrate communities in terms of species turnover to levels of EC change, considered as a proxy for human-driven salinisation, rather than observed EC. We measured species turnover, defined as change in community structure between sampling units along a spatial, temporal, or environmental gradient and typically called β-diversity (Koleff et al., 2003; Magurran, 2013; Whittaker, 1960), using the Jaccard index (JI) for invertebrate data from streams in two German states (North Rhine-Westphalia and Thuringia) following an approach of Kefford et al. (2010). Finally, we compared the response of invertebrate communities to both EC change and observed EC levels to evaluate the evidence for potential adaptation.

4.3. Methods

4.3.1. General approach

In a previous study (Le et al., 2019), we predicted current background EC in running water bodies, using a Random Forest (RF, Breiman, 2001) model driven by the major natural factors controlling salinity including geologic and soil properties, climate, vegetation, and topography. The model was trained using reference sites from governmental monitoring that were assumed to represent background EC without major human influence according to the following two criteria: (1) less than 5 % agricultural/urban land use and (2) no mining in the upstream catchments. The RF model explained 71 % of the variance in stream water EC using the natural factors outlined below. In this study, we used this model to predict the background EC in selected stream sites *(see section 4.3.2).* The difference between the predicted background EC (EC_{pred}) and the observed EC (EC_{obs}) was considered as the EC change (EC_{change}) predominantly caused by human activities. EC_{change} was calculated as:

$$EC_{change} = EC_{obs} – EC_{pred} \quad \text{(Eq4. 1)}$$

The changes in EC were split into predefined EC gradient categories (Table 4.1). We applied an approach of Kefford et al. (2010) that measures the average species turnover along the gradient of EC change categories from sites of two German states (North Rhine-Westphalia and Thuringia). Insufficient data or too short gradients prohibited the analysis of additional German states. We selected the method of Kefford et al. (2010) because this involved the pooling of samples of the same EC categories, which reduces the variability due to differences in environmental factors other than EC change. Appendix A3, Fig A3.1 summarizes the complete work-flow of the taxa turnover analysis.

4.3.2. Datasets, model application and site selection

Data on observed EC and corresponding nutrients (phosphate and nitrate) in streams were provided by state authorities for the period between 2005 and 2015. EC and EC change may be correlated with other environmental stressors (e.g. land use and nutrients) that may mask or confound the ecological response to salinity. Hence, we analysed the relationships between EC change and nutrients *(see section 4.4.2)* and land use *(see in supplementary material)*. Land use information were extracted from the Authoritative Topographic-Cartographic Information System (ATKIS) for Germany (Adv, 2016).

We compiled data on climatic, geological and soil variables that are required for the prediction of background EC (Appendix A3, Table A3.1). We calculated the mean annual precipitation and air temperature, percent of calcium oxide (CaO) and sulfur (S) in rock, soil depth, soil mean organic matter content, mean hydraulic conductivity, and enhanced vegetation index (EVI) for the upstream catchment of each sampling site. The upstream catchment for each site was derived from a digital elevation model based on the multiple flow direction algorithm (Holmgren, 1994) as implemented in GRASS GIS 7 (Metz et al., 2011; Neteler et al., 2012).

We applied the established RF model (Le et al., 2019) to the dataset of climatic, geological and soil variables to predict background EC in 259 sites from two German states (North Rhine-Westphalia: 138, Thuringia: 121). We omitted 342 and 355 additional sites in North Rhine-Westphalia and Thuringia from the model because they were considered as outside of the domain of model application. In detail, we only included sites that exhibited similar environmental attributes as the reference sites used for training the RF model, to improve the accuracy and precision of the prediction. To evaluate whether the environmental attributes of a site matched those of the reference sites, we computed the environmental space of the reference sites and removed sampling sites that fell outside of this space using the *convhulln* function in the R package *geometry* (Roussel et al., 2019). For visualization of the position of sites with respect to the environmental space of the reference sites (Appendix A3 Fig A3.2), we used principal component analysis (PCA) to project the eight environmental variables including precipitation (precip), air temperature, % S and % CaO in rock, soil depth, soil organic matter content (OC), catchment hydraulic conductivity (HC) and enhanced vegetation index (EVI) into a two-dimensional space. We found no evidence for a relevant effect of spatial autocorrelation on the EC change, which was diagnosed with semivariograms calculated using the R packages *SSN* (Hoef and Peterson, 2018) and *openSTARS* (Kattwinkel and Szöcs, 2018) (Appendix A3, Fig SA3.3).

Data for invertebrates in the sampling sites for 2005 to 2015 were provided by state authorities. Invertebrate sampling was conducted according to multi-habitat sampling (AQEM, 2002) in concert to the measurement of physicochemical variables such as EC and pH. Briefly, across a representative stream stretch different substrates were sampled according to their presence in 5 % steps. In total 20 sub-samples resulting in a sampled area of 1.25 m^2 were taken using a 0.5 mm mesh size sampler. Taxa were identified at different taxonomic levels ranging from order to species according to defined standards for German monitoring and abundances given as individuals per square meter. We restricted the analysis to samples from April and May that are standard sampling periods of monitoring by the states, because most organisms are not detectable from September to February given that they occur as egg or first instar stages that are too small for morphological taxonomic identification (Tondato et al., 2010). The number of samples differed across sites, with 2 to 4 within the 10 years (2005 - 2015). To avoid undue influence of sites with a higher number of samples, we homogenized the number of samples per site to 2 for all sites through random sampling without replacement. The abundance data were converted to presence/absence data, which were used to study species turnover. Furthermore, we removed rare taxa that occurred only in a single site of a state. In total, 387 and 276 taxa were identified from 138 and 121 sampling sites in the two states North Rhine-Westphalia and Thuringia, respectively, after removal of rare taxa (Fig 4.1).

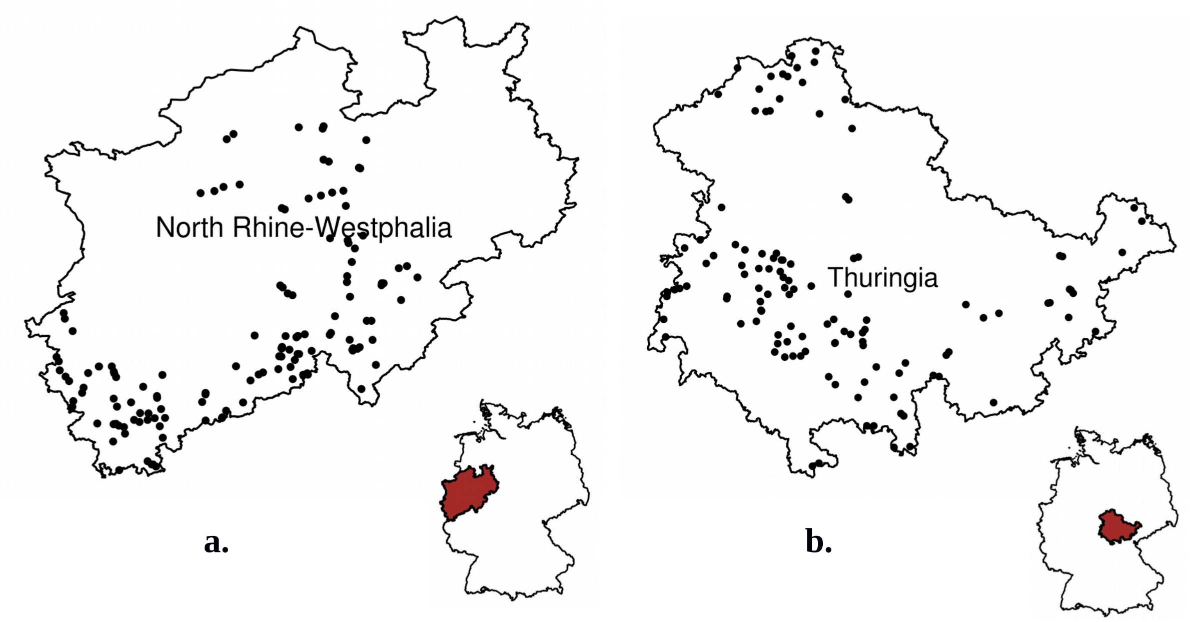


Fig 4.1: Location of monitoring sites in a. North Rhine-Westphalia b. Thuringia

4.3.3. Jaccard index

To evaluate the change in invertebrate composition along gradients of EC change, we calculated the Jaccard index (JI) between pooled sample set as in Kefford et al. (2010) (see below), for similar and different levels of EC change. We used JI to measure species turnover because it is the most widely used similarity index for assessing the compositional similarity of assemblages (Chao et al., 2004) and the least vulnerable to errors of taxonomy, enumeration, or geography (Boyce and Ellison, 2001; Schroeder and Jenkins, 2018). JI is the proportion of taxa that two sites share:

$$JI=\frac{j}{(a+b-j)} \quad \text{(Eq4. 2)}$$

where j is the number of taxa found in both sites A and B, a and b are total number of taxa in site A and B, respectively. JI ranges from 0 when both sites do not share any taxon to 1 when both have identical taxa (Whittaker, 1960). Typically, two sites exhibit a relatively low similarity (i.e. JI) due to differences in environmental variables, as well as spatial and stochastic processes influencing community composition. To increase the similarity and thereby potentially improving the detectability of the response of the community to a specific variable of interest (i.e. EC), we pooled sites of a particular EC change category to reduce the influence of environmental variables and spatial and stochastic processes following an approach of

Kefford et al. (2010). In contrast to Kefford et al. (2010), we focused on categories of EC change (i.e. difference between observed EC and background EC) instead on observed EC. However, the latter was also calculated for the purpose of comparison (Appendix A3, Table A3.2).

The minimum number of sites for an EC category in North Rhine-Westphalia and Thuringia was 6 and 5 sites, respectively, in both cases for the EC gradient 5th category [0.3 - 0.4 mS cm^{-1}). Therefore, we set the number of pooled sites termed super samples in Kefford et al. (2010) to 6 and 5 sites for North Rhine-Westphalia and Thuringia, respectively. The sites within EC categories were randomly allocated (selection without replacement) to a set of the size of the defined super sample size (Table 4.1). The JI was calculated between all pairs of pooled sample sets. Then, the mean JI between and within each category was calculated. Significant differences in JI between EC categories were assessed by "analysis of similarity" (ANOSIM). This yields the test statistic R (ranging from -1 for no differences between groups to +1 for a clear distinction of samples between groups) with a related permutation-based p-value.

Table 4.1: Number of samples and pooled sample sets per category of EC change for North Rhine-Westphalia and Thuringia

EC change category	Range of EC change [mS cm^{-1}]	Number of samples	Number of pooled sample sets	Total no of samples randomly included in pooled sample sets
		North Rhine-Westphalia		
1	< 0.05	32	5	30
2	[0.05 - 0.1)	22	3	18
3	[0.1- 0.2)	21	3	18
4	[0.2 - 0.3)	21	3	18
5	[0.3 - 0.4)	6[a]	1	6
6	[0.4 - 0.5)	22	3	18
7	>0.5	14	2	12
Total		138	20	120

EC change category	Range of EC change [mS cm^{-1}]	Number of samples	Number of pooled sample sets	Total no of samples randomly included in pooled sample sets
		Thuringia		
1	< 0.05	31	6	30
2	[0.05 - 0.1)	15	3	15
3	[0.1 - 0.2)	14	2	10
4	[0.2 - 0.3)	16	3	15
5	[0.3 - 0.4)	5[a]	1	5
6	[0.4 - 0.5)	12	2	10
7	[0.5 -1.0)	15	3	15
8	>=1.0	13	2	10
Total		121	22	110

[a] super sample size

4.3.4. Identify indicator invertebrate for EC change categories

We calculated indicator values (IndVal) (Dufrêne and Legendre, 1997) for both states to determine which invertebrate can be used as indicators of EC change categories. We used the function *multipatt* of the R package *indicspecies* (Cáceres and Jansen, 2019) to calculate the IndVal that measures the association between a taxon and an EC change category. We set the argument for the taxon-site group association in the function to '*IndVal.g'* to achieve more reliable results for unequal sample sizes between categories (De Cáceres et al., 2013). The Indval is the product of two components, called 'A' and 'B' (Cáceres and Legendre, 2009). Component 'A' (specificity value) is the proportion a taxon is found in a single EC gradient category, whereas component 'B' (fidelity value) is the proportion that taxon occurs in all sites in that EC category. The IndVal value of particular taxon for a particular EC change category ranges from 0 to 1 and reaches the maximum when the taxon only occurs in a single EC change category (high value of A) as well as in all sites of that category (high value of B).

Indicator species were selected as those that had a significant IndVal value (assessed via permutation test with 999 permutations) (De Cáceres et al., 2013; Vilches et al., 2013). We identified indicator invertebrates (IndVal*)* separately for each state and subsequently for the joint data of both states to identify indicator taxa that can be used across states.

4.4. Results and discussion

4.4.1. Range of conductivity changes

The range of EC change, the proxy for human-induced salinisation, was much higher in streams of Thuringia (range: -0.04 - 6.00 mS cm^{-1}) than in streams of North Rhine-Westphalia (-0.03 - 1.33 mS cm^{-1}). EC increased by more than 50 % over the background levels at approximately 60 % and 65 % of sites in North Rhine-Westphalia and Thuringia, respectively, whereas this proportion is 34 % for the US (Olson, 2019). This finding suggests a more homogeneous response in German rivers, which can be explained by the narrower gradients in climate and lithology compared to the US. A small fraction of sites had lower observed EC than predicted (background) values (14 % and 11 % of sites in North Rhine-Westphalia and Thuringia, respectively). However, the difference was always lower than 0.05 mS cm^{-1}. It can be explained by 1) error in predicted EC and 2) temporarily lower EC following an increased river discharge (precipitation, groundwater pumping and inter-basin transfer) (Crowther and Hynes, 1977; Olson, 2019). Conversely, we cannot rule out that individual values of high EC change, particularly in Thuringia, are due to specific geogenic conditions for which the predicted background concentrations were too low (resulting in a high difference between observed and predicted background EC).

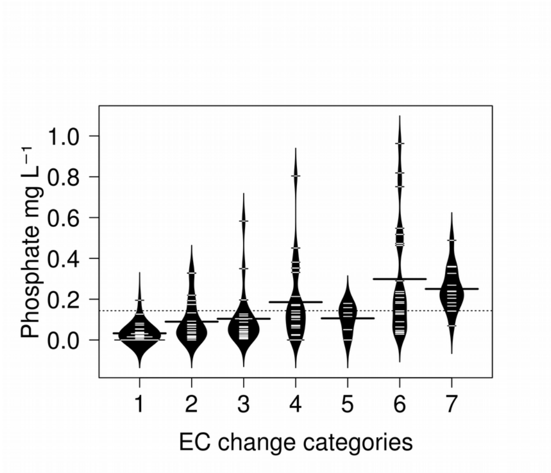
4.4.2. Nutrients along gradient of EC change categories

Freshwater ecosystems are impacted by a wide range of stressors that are often linked to land use. Among these stressors, elevated nutrient concentrations are widely occurring. An analysis for Germany found that nutrients occurred at levels above risk thresholds for ecological effects at 85 % of sites (Schäfer et al., 2016). We found that in the 10 years (2005 – 2015) considered in this study, the average nutrient concentrations recorded in streams of Thuringia ranged from below detection limit (< 0.01 mg L^{-1}) to 0.14 mg L^{-1} for phosphate and from 3.4 to 50.8 mg L^{-1} for nitrate. In streams of North Rhine-Westphalia the average nutrient concentrations ranged from below detection limit to 0.96 mg L^{-1} for phosphate and from 2.3 to 60 mg L^{-1} for nitrate. Schäfer et al. 2016 used low risk (LR) and high risks (HR) thresholds of 0.07 and 0.4 mg L^{-1} for phosphate and 2.5 and 10 mg L^{-1} for nitrate, respectively. Therefore, considering these thresholds, almost all sampling sites (99.3 %) in both states exceeded the LR threshold for nitrate, whereas approximately 40 % of the sites exceeded the LR threshold for phosphate (Appendix A3 Fig A3.4). Similarly, the HR threshold was more frequently exceeded for nitrate than for phosphate in both states (Appendix A3, Fig A3.4).

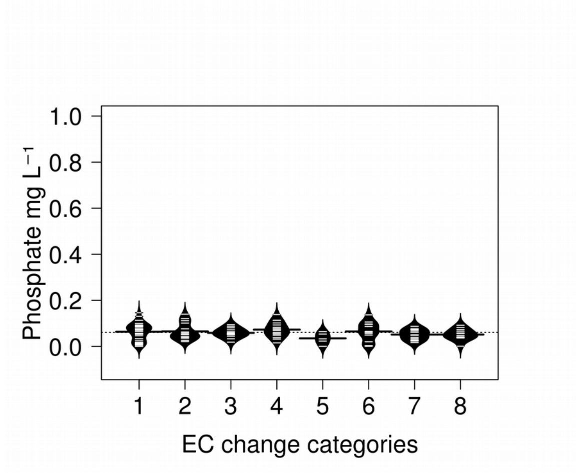
The concentrations of phosphate and nitrate increased more or less constantly with increasing category of EC change in both states North Rhine-Westphalia and Thuringia (Fig 4.2, except for the case of phosphate in Thuringia), whereas invertebrate richness decreased along the categories of EC change. Stream EC change and nitrate showed a medium positive correlation in North Rhine-Westphalia ($r = 0.58$, $p < 0.001$), whereas this relationship was rather weak in Thuringia ($r = 0.22$, $p = 0.022$). Similarly, a medium positive correlation between EC change and phosphate was observed in North Rhine-Westphalia ($r = 0.46$, $p < 0.001$), whereas this relationship was very weak in Thuringia ($r = -0.073$, $p < 0.001$). The relationship between EC and nutrients is expected because nutrients occur as ions and contribute to the conductivity.

Elevated nutrients may have contributed to the decrease in invertebrate richness and nutrients can exacerbate effects of EC on freshwater populations and communities (Alexander et al., 2016) and result in unanticipated ecological effects (Shears and Ross, 2010; Townsend et al., 2008). In a univariate analysis, Lind et al. (2018) observed that increased nutrient concentrations resulted in a higher abundance of the snail *Physa acuta* and exhibited no effects to the snail *Viviparus georgianus*. In contrast, high salt concentrations led to the extinction of *V. georgianus* but did not affect the abundance of *P. acuta*. High nutrient concentration in combination with increased salinity in the river Lippe caused physiological stress on the indigenous invertebrate communities, making them vulnerable and prone to invasive species such as *Corbicula fluminea*, *Gammarus tigrinus*, *Dikerogammarus villosus*, *Potomopyrgus antipodarum* and *Hypania invalida* (Schröder et al., 2015). Besides, high nutrient concentrations in combination with increased salinity can also promote algal blooms

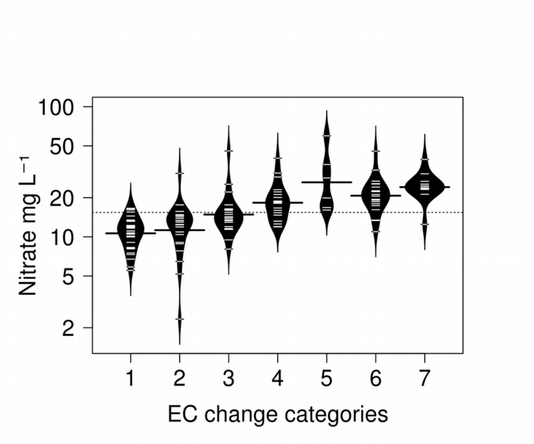
(Schulz and Cañedo-Argüelles, 2019), leading to the dominance of grazers and scrapers (Brucet et al., 2010; Jeppesen et al., 2015, 2007). This results in a different composition of invertebrate functional feeding groups (Kefford et al., 2012) regardless of the salinity in the rivers, thereby obscuring causality between salinity and community composition. A range of measures could be applied to reduce the effects of eutrophication and salinisation on streams, thereby benefitting biodiversity. These measures comprise managing the direct impacts of native vegetation loss, conserving or restoring the riparian vegetation to regulate light and temperature and restoring or managing hydrological variability to dilute the concentrations of nutrients and other salts.



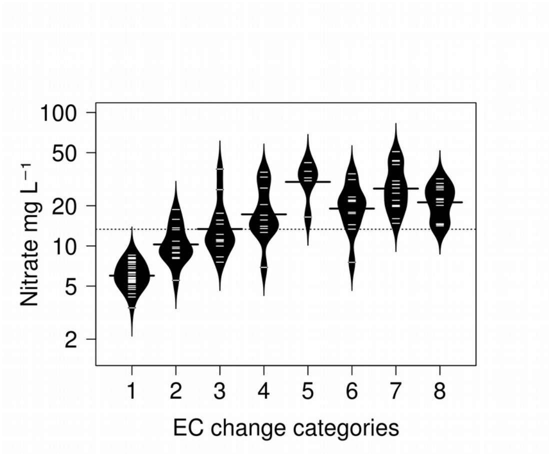
a.



b.



c.

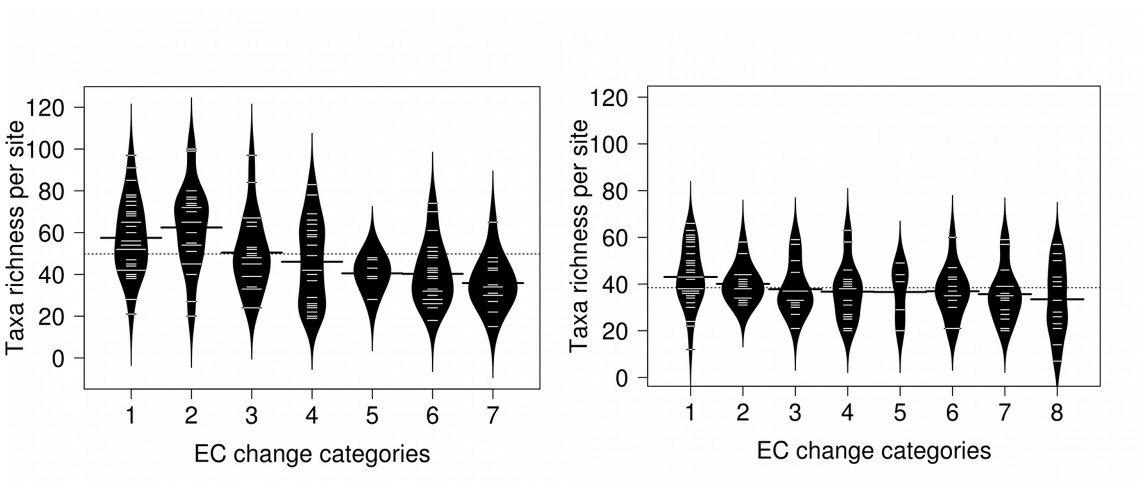


d.

Fig 4.2: Nutrients across EC change categories in North Rhine-Westphalia (a, c); Thuringia (b, d). White lines represent observations, where the length of the line indicates the relative frequency. The solid black line gives the mean value per group, the dashed black line gives the mean value over all groups

4.4.3. Invertebrate richness along the gradient of EC change categories

In the 10 years considered here (2005 - 2015), an average of approximately 50 and 38 different taxa were recorded from each individual site in North Rhine-Westphalian and Thuringian rivers, respectively. Generally, a higher EC change was associated with a reduction in taxa richness in both states (Appendix A3, Fig A3.5). The average invertebrate richness per site dropped along the gradient of EC change categories from 60 to 37 and from 43 to 35 in North Rhine-Westphalia and Thuringia, respectively (Fig4. 3a & 4.3b). This is in agreement with previous studies suggesting that an increase in EC (in our case increasing EC change) reduces the taxa richness such as invertebrates (Braukmann and Böhme, 2011; Cañedo-Argüelles et al., 2013; Schröder et al., 2015). For instance, Kefford et al. (2011) found approximately 13 %, 4 % and 20 % species loss in all taxa, crustaceans and molluscs, respectively, for a 30 % increase in observed EC in a large-scale study in Southeast Australia. The taxa richness, excluding halophilic species, decreased dramatically as salinity exceeded 2.6 TDS g L^{-1} (~ 4.3 mS cm^{-1}) in the wheat-belt region of Western Australia (Pinder et al., 2005). Conversely, the taxa richness at freshwater monitoring sites at Breitungen stream (Thuringia) increased from 20 to 38, during the period of decreasing salt contamination from mining activities in the 1990ies, and 62 taxa were identified in 2008 (Bäthe and Coring, 2011). Finally, increasing EC is typically associated with a change in invertebrate composition through 1) non-compensated loss of single taxa because of increasing osmoregulatory effort and associated energy demand (Cañedo-Argüelles et al., 2013), 2) compensated loss, i.e. shifts from salt-sensitive to salt-tolerant invertebrates adapted to increased salinity levels (Braukmann and Böhme, 2011; Piscart et al., 2005; Schröder et al., 2015), and 3) shifts in the food web resulting in a different composition of invertebrate functional feeding groups, and consequently species, (Kefford et al., 2012).



a. **b.**

Fig 4.3: Taxa richness per site at two sampling occasions in a. North Rhine-Westphalia, b. Thuringia. White lines represent observations, where the length of the line indicates the relative frequency. The solid black line gives the mean value per group, the dashed black line gives the mean value over all groups

4.4.4. Taxa turnover along the gradient of EC change/observed EC categories

EC change, the proxy for human-induced salinisation, correlated strongly positively with observed EC in both states (North Rhine-Westphalia: correlation coefficient $r = 0.97$, $p < 0.001$; Thuringia: $r = 1.0$, $p < 0.001$). The observed EC level is strongly influenced by geological and climatic factors (Bonte and Zwolsman, 2010; Jackson and Funk, 2019; Jeppesen et al., 2015; McNeil and Cox, 2007; Nielsen and Brock, 2009; Reimann et al., 2009; Rothwell et al., 2010) and we hypothesised that invertebrate communities may be adapted to this natural background levels in EC and more strongly respond to anthropogenic EC change. However, given the relatively small variability in EC change and that EC change and observed EC correlated very strongly for our study regions, it is not surprising that the responses to EC change and observed EC were very similar (see below). However, in other regions or at larger scales where the background EC exhibits a stronger variation, and the correlation between EC change and observed EC is weak, EC change may provide a more reliable relationship with the invertebrate community alteration than the observed (absolute) EC. This could be, for instance, regions with a wide distribution of naturally saline streams such as in Southern Europe, USA and Australia (e.g. Gutiérrez-Cánovas et al., 2019)

We found a decrease in mean JI(s) along the gradient of EC change (North Rhine-Westphalia: ANOSIM Global $R = 0.41$, $p = 0.003$; Thuringia: $R = 0.70$, $p = 0.001$) and observed EC categories (North Rhine-Westphalia: $R = 0.19$, $p = 0.025$; Thuringia: $R = 0.60$, $p = 0.001$). The JI(s) between the EC change categories were generally greater than 47 %, with a maximum of approximately 70 % as change of EC and observed EC exceeded 0.4 mS cm^{-1} and 0.5 mS cm^{-1}, respectively, in both German states (Table 4.2 and 4.3), and when compared to categories of low EC change. Very similar results were obtained for observed EC categories (Appendix A3, Table A3.3 and A3.4). Several other previous studies focused on changes in invertebrate communities in response to the observed EC with considerable variation between studies. A threshold of observed EC value exceeding 0.9 mS cm^{-1} was suggested for the lower and middle course of the Lippe River, Western Germany, where major changes in the macroinvertebrate composition were observed (Schröder et al., 2015). Piscart et al. (2005) observed the disappearance of several taxa as salinity exceeded

1.4 g L^{-1} (~2.0 mS cm^{-1}) at four sites along a small permanent salinity gradient (0.28 - 3.4 mS cm^{-1}) in the Meurthe River, Northeastern France. Pinder et al. 2005 found a dramatic decline in species richness, excluding halophilic species, at a wetland Western Australia as salinity exceeded 2.6 TDS g L^{-1} (~ 4.3 mS cm^{-1}). These thresholds are different from ours partly because we focused on EC change levels, whereas absolute EC was considered in previous studies. Variability in the EC thresholds between studies can be explained by differences in the considered community responses (e.g. community change, species loss, or species turnover), differences in the considered species pools that are shaped by local and regional environmental factors and biotic relationships (e.g. competition, predator avoidance) as well as interactions with other stressors. Such interactions of salinity with other stressors (e.g. water temperature (Hopkins et al., 2017; Kennedy et al., 2004), lime content (Soucek and Kennedy, 2005), pH-value (Dunlop et al., 2005), nutrients (Lind et al., 2018) and other toxicants can modify salinity tolerance of taxa (Velasco et al., 2019). Moreover, saline toxicity in freshwater organisms depends on ionic composition and ratios, not just total salinity (Cañedo-Argüelles et al., 2016) since different ions have different toxicities. For example, among the various ions, K^+ seems to be the most toxic to freshwater fauna (Griffith, 2017); SO_4^{2-} seems to be more toxic than Cl^- and Na^+ (Carbonell et al., 2012; Céspedes et al., 2013; Zalizniak et al., 2006). Kefford et al. (2004) found that several species of macroinvertebrates, including the snail *Physella acuta*, were more sensitive to pure sodium chloride than to a salt mixture containing additional ions to sodium chloride at comparable salinity levels, thus ions other than Na^+ and Cl^- decreased the toxicity of water. Survival of the ephemeroptera *Centroptilum triangulifer* and the clam *Lampsilis siliquoidea* was reduced at elevated Mg^{2+}, Ca^{2+}, K^+, SO_4^{2-}, and HCO_3^-, yet *C. triangulifer* was uneffected at elevated Na^+, K^+, SO_4^{2-}, and HCO_3^- at comparable conductivity (Kunz et al., 2013).

Table 4.2: The mean Jaccard index (JI) of invertebrates similarity between EC change categories North Rhine-Westphalia. Values along the diagonal represent the mean JI(s) of categories, i.e. within-category variability

EC change categories	Range of EC change	1	2	3	4	5	6	7
		< 0.05	[0.05- 0.1)	[0.1- 0.2)	[0.2- 0.3)	[0.3- 0.4)	[0.4- 0.5)	>=0.5
1	< 0.05	0.51						
2	[0.05- 0.1)	0.52 (-0.22)	0.52					
3	[0.1- 0.2)	0.48 (0.35)	0.51(-0.22)	0.47				
4	[0.2- 0.3)	0.45 (0.41)	0.48 (0.07)	0.46 (-0.15)	0.42			
5	[0.3- 0.4)	0.42 (0.88)	0.46 (1.0)	0.45 (0.33)	0.46 (-0.33)	NA		
6	[0.4- 0.5)	0.28 (0.90[a])	0.31 (0.52)	0.34 (0.41)	0.32 (0.44)	0.36 (-0.33)	0.33	
7	>= 0.5	0.23 (0.99[a])	0.26 (0.83)	0.28 (0.83)	0.28 (0.66)	0.33 (0.0)	0.36 (-0.21)	0.31

Global R = 0.41, p = 0.003
In bracket: pairwise ANOSIM statistic R
[a]pairwise ANOSIM p value < 0.05
NA: undefined (a result of only one pooled set)

Table 4.3: The mean Jaccard index (JI) of invertebrates similarity between EC change categories Thuringia. Values along the diagonal represent the mean JI(s) of categories, i.e. within-category variability

EC change categories	Range of change of EC	1	2	3	4	5	6	7	8
		<0.05	[0.05- 0.1)	[0.1- 0.2)	[0.2- 0.3)	[0.3- 0.4)	[0.4- 0.5)	[0.5- 1.0)	>=1.0
1	< 0.05	0.50							
2	[0.05- 0.1)	0.47 (0.32)	0.50						
3	[0.1- 0.2)	0.44 (0.61)	0.48 (0.25)	0.50					
4	[0.2- 0.3)	0.38 (0.94)	0.42 (0.70)	0.49 (-0.42)	0.45				
5	[0.3- 0.4)	0.30 (1.00)	0.37 (1.00)	0.39 (1.00)	0.45 (0.11)	NA			
6	[0.4- 0.5)	0.29 (1.00[a])	0.34 (1.00)	0.42 (1.00)	0.41 (0.5)	0.44 (1.00)	0.53		
7	[0.5-1.0)	0.27 (1.00[a])	0.32 (1.00)	0.37 (0.67)	0.39 (0.59)	0.44 (-0.55)	0.45 (-0.33)	0.42	
8	>=1.0	0.25 (1.00[a])	0.29 (1.00)	0.35 (1.00)	0.37 (0.75)	0.43 (0.0)	0.41 (0.5)	0.44 (-0.16)	0.46

ANOSIM Global R = 0.70, p = 0.001
In bracket: pairwise ANOSIM statistic R
[a] pairwise ANOSIM p value < 0.05
NA: undefined (a result of only one pooled sample set)

For the same EC change or observed EC categories, the JI(s) between the pooled sample sets were similar (JI ~ 0.50), which suggests that the variability across sample sets was similar within different categories. Hence, although the sample sets were from the same EC categories, they exhibited approximately 50 % species turnover, similar to results observed in Kefford et al. (2010). This can be explained by pooling samples that were, although similar in terms of EC change, reflecting a wider range of environmental factors, spatial and stochastic processes (Kefford et al., 2010), and ionic composition (Braukmann and Böhme, 2011; Cañedo-Argüelles et al., 2016; Potapova and Charles, 2003). Increasing the number of samples in pooled sample sets (super sample size) would result in greater similarity within the EC categories (Kefford et al., 2010). However, insufficient data prohibited the definition of a larger super sample size (only 5 and 6 samples in our case). Notwithstanding, this super sample size allowed to detect a significant turnover in species composition. Besides, applying more classical approaches such as removing rare species would increase the average similarity within categories. However, we decided to also consider the rare species in our analysis as this may still provide interesting information on sensitive taxa.

The JI(s) between categories were approximately similar for observed EC and for EC change, which could be expected, given the strong correlation between EC change and the observed EC. However, we found that the JI(s) between categories were higher for observed EC than for EC change in 10 of 13 cases (Table 4.4). This result is in line with our hypothesis that invertebrate communities may be adapted to natural background levels of EC and respond to anthropogenic EC change. Given potential adaptations, the communities may relate stronger to EC change than to the observed EC, in particular where the observed EC is mainly driven by the background EC. The mechanism behind this adaptation is likely to be complex and based on phenotypic plasticity as well as genetics and epigenetics (Cañedo-Argüelles et al., 2016; DeFaveri and Merilä, 2014; Kozak et al., 2014). For example, Posavi et al., (2014) observed genetic variation in salinity tolerance in *E. affinis* populations that allowed rapid adaptation to salinity changes during habitat invasions. Populations of *D. magna* have been documented to inhabit both fresh and slightly salty waters (Teschner, 1995), with strong evidence of phenotypic plasticity in terms of ability to adjust osmoregulation to cope with various levels of salinity (Martínez-Jerónimo and Martínez-Jerónimo, 2007).

Table 4.4: Comparison of Jaccard index (JI) between observed EC and EC change categories

Categories	Range of EC change/observed EC	Observed EC /EC change categories			
		[0.1-0.2)	[0.2- 0.3)	[0.3- 0.4)	[0.4 -0.5)
		North Rhine-Westphalia			
		3	4	5	6
3	[0.1-0.2)	0.47 (0.53)			
4	[0.2- 0.3)	0.46 (0.51)	0.42 (0.48)		
5	[0.3- 0.4)	0.45 (0.48)	0.46 (0.50)	NA (0.51)	
6	[0.4- 0.5)	0.34 (0.50)	0.32 (0.51)	0.36 (0.52)	0.33 (NA)
		Thuringia			
			4	5	6
4	[0.2- 0.3)		0.45 (0.45)		
5	[0.3- 0.4)		0.45 (0.43)	NA (0.45)	
6	[0.4- 0.5)		0.41 (0.42)	0.44 (0.45)	0.53 (0.39)

In bracket: The mean Jaccard's index (JI) of similarity between observed EC categories; NA: undefined (a result of only one pooled sample set)
[a] categories for EC change and observed EC share the same range values

4.4.5. Indicator taxa

Indicator value (Indval) analyses resulted in 67 (17 % of total identified taxa) and 45 (16 %) invertebrates selected to indicate the EC change categories in North Rhine-Westphalia and Thuringia, respectively (Appendix A3, Table A3.5, A3.6). The differences in salinity change between taxonomic groups were in agreement with previous studies: Crustacea, Gastropoda and Amphipoda were the most tolerant groups (Piscart et al., 2005; Szöcs et al., 2014) whereas Ephemeroptera, Plecoptera, and Trichoptera were the most sensitive groups (Clements and Kotalik, 2016; Halse et al., 2003; Kefford et al., 2012; Schröder et al., 2015; Szöcs et al., 2012). We found that communities at sites with higher EC change were dominated by the amphipods *Gammarus tigrinus, Chelicorophium curvispinum* and the snail *Potamopyrgus antipodarum*. These results are consistent with previous studies (Arle and Wagner, 2013; Szöcs et al., 2014) for the River Werra, Germany. Piscart et al. (2011, 2005) also observed a similar pattern along a salinity gradient in the Meurthe River, northeastern France. In their study, the invasive species *G. tigrinus, P. antipodarum* and *C.*

curvispinum were more abundant at high EC sites due to increased salt ions during the period of intense mining over the last decades. Besides, high salinity tolerance of these invasive species is indeed required to survive during ballast water exchanges from freshwater to seawater (Piscard et al., 2011). However, while *G. tigrinus* and *P. antipodarum* are tolerant to salinity, the strong increase could also be an indirect effect, i.e. the reduction of more sensitive competitors (Piscart et al., 2011).

Only 13 invertebrates that were selected as indicator taxa matched across both states (Appendix A3, Table A3.7). This can be explained by the fact that many invertebrates (42 %) were present in North Rhine-Westphalia but absent or rare in Thuringia (e.g. *Hypania invalida* or *Ecdyonurus venosus*). Ten of the 13 invertebrates that overlapped across both states were significantly associated with the low EC change categories 1st or 2nd. Of the 13 species, only *P. antipodarum* indicated sites with a high change of EC (> 0.5 mS cm^{-1}) in both North Rhine-Westphalia and Thuringia. The mayflies *Ephemera danica* and *Rhithrogena semicolorata* were indicators of the 2nd EC change category ([0.05- 0.1) mS cm^{-1}) in North Rhine-Westphalia, but of the 4th ([0.2- 0.3) mS cm^{-1}) and 5th ([0.3- 0.4) mS cm^{-1}), respectively, in Thuringia. This may indicate tolerance of these invertebrate species to moderate salinity levels in streams of Thuringia. However, a general analysis of salinity preferences is challenging, because studies often yielded inconsistent results. For instance, we found the ephemeroptera *Epeorus assimilis* and the plecopterans *Protonemura sp.* and *Leuctra sp.* as indicators of low salinity levels, which matches results by Feeley and Kelly-Quinn, (2015), Short et al. (1991), Timpano et al. (2018), whereas Short et al. (1991), Wichard et al. (1972) found these taxa occurring at a much wider range of salinity levels. These differences partly appear to be due to ecological properties that vary between taxa, the individual plasticity of organisms (Schröder et al., 2015), historical salinity exposure of species (Sala et al., 2016), and regional differences including the occurrence of stressors and biotic competitors (Velasco et al., 2019). This makes it difficult to describe a uniform response of freshwater invertebrates to salinity levels in water.

4.5. Conclusions

The response of invertebrates along the gradient of EC change categories represents a novel approach to identify the effects of human-induced salinisation on freshwater invertebrate composition and may contribute to the identification of the development of adaptations. This approach could be applied for other stressors and to other types of ecosystems where background levels can be defined. Analyses of the response of invertebrates to individual stressors (EC change in our case) are, however, subject to certain limitations due to frequent co-occurrence with other stressors in the water, potentially leading to interactive effects and obscuring causality between stressor levels and responses. Therefore, adopting a multiple stressor approach is an imperative for an integrated ecological and ecotoxicological assessments of rivers.

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Chapter 5: General discussion and outlook

5.1. WWTP effluents are important sources of pesticides in freshwater

In chapter 2, we found a significantly higher total pesticide toxicity in terms of logarithm-transformed toxicity index values for sites with a WWTP than sites without WWTPs. This result shows that WWTP effluents represent a point source of pesticides in small agricultural streams on a large spatial scale in Germany. Similar results were found by other previous studies, yet most studies on this topic were limited in scale (a small number of WWTPs on the local or regional scale) and the number of pesticides considered. For example, Münze et al. (2017) observed that seven WWTP effluents in the countryside and suburbs of central Germany significantly increased insecticide and fungicide concentrations in water intake. Similarly, Bunzel et al. (2013) found 75 % of the sampled sites with a WWTP within 3 km upstream had insecticidal effects on the structure of the macroinvertebrate community in streams in Hesse, Germany. Expanding on the previous investigations, we did our study in small agricultural catchments as pesticide toxicity contributed from WWTPs in small streams has been previously reported to be generally higher compared to larger rivers (Lorenz et al., 2017; Schulz, 2004). Similarly, a recent study by Neale et al. (2017) also found that wastewater effluents were relevant sources of pesticides in small water bodies. For future large-scale studies on this topic, it is recommended to collect data on other relevant parameters, such as agricultural practices, treatment efficiency, and the size of WWTPs (population equivalents) to resolve the over- or under-estimation of the contribution of WWTPs' toxicity. This information can be gained by surveying the local WWTPs.

Concerning the toxicity of different pesticide groups, we observed that herbicides contributed most to total pesticide toxicity with the most frequently detected being diuron, terbuthylazine, isoproturon, terbutryn, and metazachlor. Similarly, WWTPs are known to introduce large quantities of herbicides to bodies of water from many previous studies. Neumann et al. (2002) observed that dominant pesticides detected in the WWTPs effluent were herbicides with the almost continuous presence of atrazine, ethofumesate, terbuthylazine, chloridazon, and metamitron. In a study of 24 medium-sized streams affected by WWTP inputs in catchments across three Swiss biogeographical regions (Swiss Plateau, Jura, and Pre-alps), Kienle et al.

(2019) observed that WWTPs were an important point source of herbicidal compounds discharging to surface water. In the catchment area of Lake Greifensee, 65 %, 14 %, 28 %, and 18 % of the total input of the herbicides mecoprop, atrazine, metolachlor, and isoproturon, respectively, originated from WWTPs (Gerecke et al., 2002). The herbicides phenylurea and glyphosate were frequently detected in the effluent of WWTPs in Switzerland (Gerecke et al., 2002; Poiger et al., 2020). However, the relative contribution of herbicides to toxicity should be interpreted with caution. Pesticide monitoring in small streams relying on grab sampling, largely disconnected from precipitation events, underestimated pesticide toxicity, particularly of hydrophobic insecticides and fungicides (Stehle et al., 2013; Szöcs et al., 2017). Automatic event-driven samplers and passive samplers may help overcome this shortcoming and assist the reliable interpretation of the contribution of different pesticide groups to total pesticide toxicity. Besides, more herbicides were measured in total and per sample, which may contribute to higher toxicity for herbicides. Future monitoring of small water bodies should also capture precipitation events, which agrees with other studies, such as Lorenz et al. (2016), and the number of measured compounds should be compared to the number of applied herbicides, insecticides, and fungicides.

Additional treatment steps, such as ozonation, adsorption to activated carbon (Hernández-Leal et al., 2011; Margot et al., 2013; Reungoat et al., 2010), or advanced oxidation processes (Hollender et al., 2009), were suggested to remove not only pesticides but also other organic micropollutants (e.g., pharmaceutical residues, and cosmetic ingredients or detergents) from municipal WWTP effluents. A multi-year study found a decrease in the ecological quality downstream from a WWTP and observed a recovery of the community of the small stream Furtbach, after the implementation of an ozonation treatment step (Ashauer, 2016). Similarly, Bundschuh et al. (2011) found an increase in the number of sensitive taxa and in ecosystem functioning in terms of leaf decomposition, from the implementation of the same treatment technique. However, implementation of additional treatment steps in WWTPs is controversial due to a high level of investment and increased maintenance costs that may outweigh the benefits, especially in agricultural areas with low population densities.

5.2. Implications and challenges of the predictive natural background salt ion model

In chapter 3, I set up two models, a multiple linear regression and a random forest, to account for natural spatial variation of salt ions (Ca^{2+}, Mg^{2+}, SO_4^{2-} and EC) in water without considering human-induced impacts. These models contribute to establishing appropriate background conditions that can be applied to assess whether the freshwater community has deteriorated through secondary salinisation in the case of EC change investigated in chapter 4. Additionally, the established models predicted increasing trends in background EC for around 80 % of the streams in Germany under different scenarios of future climate change. This finding suggests potential risks in other Central European regions with similar lithologic and climatic properties. Variables (such as the percentage of crops or developed land) can be added in the models to estimate the salt ions and EC with the presence of anthropogenic land use and then to calculate the range of alteration between background salinity and salinity caused by human land use. Such models were used to assess the relative impacts on streams' salinity (EC in our case, chapter 3) due to future climate change, future land use change, and both combined (Olson, 2019).

Background freshwater salinisation is the result of interactions between diverse hydrological, geochemical, and biological processes controlled by natural determinants: lithology, climate, vegetation, relief, and soil properties (Olson and Hawkins, 2012; Reimann et al., 2009; Riebe et al., 2003; Rothwell et al., 2010; Stallard and Edmond, 1983; Viers et al., 2009). The level of the relative contribution of these factors to the water chemistry in rivers depends not only on the distribution of different hydrochemical processes and the seasonal variations of precipitation/temperature (Last, 1992), but also on the size, elevation of the watersheds, and the local differences in surface and groundwater (Last, 1992; Pratt and Chang, 2012; Rothwell et al., 2010; Woocay and Walton, 2008). However, obtaining geological data presents a challenge due to the uncertainties of geologic feature classifications such as individual rock samples representing an entire area, and discrete rock layers converted into a series of geochemical description maps as continuous variables. Although geologic data was created by applying these approaches, they may not fully account for the chemical variation among layers resulting from different rocks types within a layer. Futhermore, the uncertainties of predictions for precipitation and temperature patterns are subject to certain limitations of predictive background models (McNeil and Cox, 2007). Natural salinisation processes interacting with human processes can exacerbate predicted background salinity in water. For example, acid rain accelerates chemical weathering due to chemical dissolution and ion exchange in rocks and soils (Guo et al., 2015; Kaushal et al., 2013) and then contributes to increased concentrations of major ions in water, including bicarbonate, calcium, magnesium, and potassium (Aquilina et al., 2015; Cañedo-Argüelles et al., 2017; Palmer et al., 2010). Besides the omission of atmospheric deposition and extrapolating statistical relationships beyond the range of observed climate data (see detail in section 3.4.3) there are other limitations of the predictive model. Consequently, models may over or underestimate the increase of natural background salinity in freshwater.

5.3. The toxicity of salinity on invertebrate turnover, and interactions between salinity and other stressors in freshwater

We found that invertebrate turnover between EC gradient categories is generally greater than 47 %, with a maximum of approximately 70 % in sites with EC more than 0.4 mS cm^{-1} change compared to the background EC (chapter 4). This finding supports previous studies emphasising that increases in EC reduce the taxa richness of taxa such as invertebrates (Braukmann and Böhme, 2011; Cañedo-Argüelles et al., 2012; Pinder et al., 2005; Piscart et al., 2005; Schröder et al., 2015). However, the frequent co-occurrence with other stressors potentially leads to interactive effects, obscuring the causality between stressors and responses (Jackson et al., 2016; Ormerod et al., 2010; Velasco et al., 2019). Therefore, analyses of the response of freshwater organisms (invertebrates in our case) to individual stressors (EC change in our case) are subject to certain limitations. Williams (1987) stated that in agricultural regions pesticides often co-occur with increased salt ions concentrations, thus pesticides and salinity can potentially interact to impact freshwater organisms. Salinity influences the distribution of polar pesticides between water and sediment or suspended particles, and thus may influence the effects of pesticide effects on water organisms, according to Saab et al. (2011). However, no interaction between the two stressors was found by Schäfer et al. (2012, 2011) or Szöcs et al. (2012), suggesting that their combined effects might largely be additive. Additionally, salt-heavy metals together exacerbated the effects of salinity on aquatic organisms. Mahrosh et al.

(2014) observed that increased concentrations of heavy metals and increased salinity in combination was more severe than the effects of individual stressors on the development of salmon (*Salmo salar*) eggs. Another study on the combined effects of cadmium and salinity on juvenile *Takifugu obscurus* observed that the juveniles could survive well under different salinities; however, with Cd exposure, the survival rates significantly decreased (Wang et al., 2016). Nutrients occur as ions, and contribute to the salinity concentration in freshwater, and exacerbate the effects of salinity on populations and communities in water (see more details in section 4.4.3). On the contrary, salts can mobilize organic nitrogen, ammonium, and phosphorus via ion exchange and stimulate eutrophication (Duan et al., 2012; Duan and Kaushal, 2015; Haq et al., 2018; Kaushal et al., 2017, 2013). Eutrophication promotes the proliferation of cyanobacteria blooms, not only due to the direct effects of salts on algae (Cañedo-Argüelles et al., 2017), but also because of the reduction of salt sensitive cladocerans, which feed on algae (Brucet et al., 2010; Jeppesen et al., 2015, 2007). The effect of salinity combined with other environmental factors, such as temperature (Bœuf and Payan, 2001; Hall and Burns, 2001; Hopkins et al., 2017; Kennedy et al., 2004), hardness (Kennedy et al., 2005), pH (Zalizniak et al., 2009), on freshwater organisms were investigated in previous studies.

Salinity can interact or combine with other stressors that can confound the ecological response to salinity in water. We recommend that future research efforts concentrate more on the ecotoxicological effects of multiple stressors to better understand the threats of salinity to organisms and communities in freshwater. *S*aline toxicity in freshwater organisms relies on ionic properties, composition, and ratios of salts, not just the total salt concentration (Cañedo-Argüelles et al., 2016) since not all ions are equally toxic to freshwater organisms. For example, among the different ions, K^+ seems to be the most toxic to freshwater fauna (Griffith, 2017). The individual ion acute toxicities to species *Ceriodaphnia dubia*, *D. magna*, and *Pimephales promelas* were summarized as $K^+ > HCO_3^- \approx Mg^{2+} > Cl^- > SO_4^{2-}$, with no significant effects from either Na^+ or Ca^{2+} (Mount et al., 1997). On the contrary, Soucek & Dickinson, 2015; Clements & Kotalik, 2016 observed that SO_4^{2-} seems to be more toxic than Cl^-. Mg^{2+} can be toxic at concentrations close to natural background levels, but the toxicity of Mg^{2+} depends on Ca^{2+} concentrations. Ca^{2+} deficient waters pose the greatest risk to aquatic life with exposure to very low ionic concentrations (van Dam et al., 2010). Similarly, Mount et al. (2016) observed that the Ca^{2+} concentration was the factor influencing the toxicities of Na^+ and Mg^{2+} salts, whereas the toxicities of K^+ salts depended on the concentration of Na^+. Water hardness can ameliorate the toxic effects of other ions (Elphick et al., 2011; Soucek et al., 2011). Dwyer et al. (1992) found that the survival of Morone saxatilis increased as hardness increased at comparable salinity levels. The acute toxicity of SO_4^{2-} to an amphipod *Hyalella azteca*, decreased with increasing concentrations of 5 mg to 25 mg Cl L^{-1} (Soucek, 2007), and with an increasing Ca:Mg ratio at the comparable hardness of 100 mg L^{-1} (Davies and Hall, 2007). K^+ could ameliorate SO_4^{2-} toxicity in cladocerans, midges, mussels, and fish (Wang et al., 2016). Kefford et al. (2004) observed that the snail *P. acuta*, was more sensitive to pure sodium chloride than to a salt mixture containing additional ions added to sodium chloride at comparable salinity levels, thus ions other than Na^+ and Cl^- decreased the toxicity of the water. Ionic proportions of the total measured salinity can vary across space and time leading to different toxicities of salinity in individual freshwater organism. We suggest that future research efforts investigate how the ionic composition of salinity affects species.

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APPENDIX A1

Table A1.1 Toxicity values of neonicotinoid pesticides (ECOTOX - US EPA, 2016)

Compound	*Species*	Concentration (μg/L)
Acetamiprid	*Chironomus riparius*	20.9
Thiacloprid	*Baetis rhodani*	4.6*
Imidacloprid	*Simulium vittatum*	9.54
Thiamethoxam	*Chironomus sp.*	35
Clothianidin	*Chironomus sp.*	22

* LC_{50} for 96h

Table A1.2 Examples for pesticide removal efficiency in WWTPs reported in selected studies

Category	Compound	Region	Influent (μg L^{-1})	Effluent (μg L^{-1})	Removal[b] (%)	References[a]
Herbicide	Atrazine	Europe	0.02-28	0.004-0.73	0-25	1, 2, 3, 6
	Diuron	Europe	0.03-1.96	0.002-2.53	26.7-71.9	1, 2, 3, 6
Insecticide	Diazinon	Europe	<0.68	0.0007-4.16	0	1, 2, 3, 6
	Clotrimazole	Europe	0.012-0.08	ND-0.005	84.5-93.6	3, 4, 5
Fungicide	Tebuconazole	Greece, Spain, Swiss	ND[c]-1.89	0.0005-0.69	0-58.7	1, 4, 5

[a] 1.(Campo et al., 2013); 2. (Köck-Schulmeyer et al., 2013); 3. (Loos et al., 2013); 4. (Kahle et al., 2008); 5. (Stamatis et al., 2010); 6. (Singer et al., 2010).

[b] Removal efficiencies were calculated in study of Luo et al, 2014.

[c] ND: not detected

Appendix A1

References

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APPENDIX A2

Table A2.1 Attributes assigned for each unit of lithology.

Lith	%CaO	%MgO	%S	Uniaxial Compressive Strength (Mpa)	Geometric Mean Hydraulic Conductivity (µm/sec)
Amphibolite	9.9000	6.7000	0.0244	181.9100	0.0016
Andesite	6.5000	3.5000	0.0140	128.5600	0.2380
Anhydrite	39.3000	0.3000	58.7014	89.8300	0.0018
Aplite	0.8000	0.2000	0.0200	136.0800	0.0045
Arkose	1.3500	0.5400	0.0142	130.9300	0.0054
Basalt	9.3000	6.5000	0.0300	193.8200	0.6070
Basaltic-andesite	8.2000	4.1000	0.0050	55.4000	0.0551
Biotite-gneiss	2.0000	0.6000	0.0050	152.2000	0.0253
Biotite-schist	2.3000	1.7000	0.6900	68.8000	0.2500
Black-shale	0.5000	0.4000	1.7900	19.0500	0.0001
Boulders	0.4500	0.4900	0.0200	5.0000	106.0000
Calcarenite	54.3600	0.6700	0.0471	17.6500	0.4510
calcareous-	27.3600	1.1200	NA	NA	NA
Conglomerate	21.1400	8.4600	NA	NA	NA
calcareous-Sandstone	13.9000	4.5000	NA	NA	NA
calcareous-Siltstone	15.5000	3.5000	0.0200	97.4000	0.3800
Calc-silicate	47.7800	0.5900	0.0459	76.5600	0.1400
Carbonate	53.4000	0.3000	0.0190	18.9800	0.0013

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Lith	%CaO	%MgO	%S	Uniaxial Compressive Strength (Mpa)	Geometric Mean Hydraulic Conductivity (µm/sec)
Chalk	0.5000	1.8000	0.0385	0.3300	0.0011
Clay	0.9700	2.2000	0.2700	16.1500	0.0000
Claystone	0.6100	0.6400	0.0400	103.1000	0.0159
Conglomerate	1.0600	0.8200	0.0350	95.6000	0.0846
Conglomerate-sandstone	0.0000	0.0000	0.0200	90.7800	0.1270
	6.3000	3.2000	0.0600	226.1400	0.0013
Dacite	2.8900	2.9000	NA	NA	NA
Diorite	28.2000	13.4000	NA	NA	NA
dolomitic-Claystone	23.5000	3.1000	NA	NA	NA
dolomitic-Limestone	0.0000	0.0000	0.0320	138.2000	0.0870
dolomitic-Marlstone	11.5000	6.9000	0.0120	400.0000	0.0042
Dolostone	10.8000	4.1000	0.1123	43.4000	1.3900
Eclogite	9.7000	6.5000	0.0600	224.0100	0.7040
Foidite	2.3000	1.1000	0.0100	152.2100	0.0253
Gabbro	0.0000	0.0000	0.0300	188.6800	0.0001
Gneiss	3.4000	1.4000	0.0070	135.6500	0.0012
Granite	9.9000	6.6000	0.0280	214.6700	0.2910
Granodiorite	0.4100	0.4800	0.0208	2.2900	8.3000
Granulite	0.8400	1.8100	0.0058	81.8800	0.0137
Gravel	8.5000	6.7000	0.1586	49.8100	0.0086
Graywacke	31.3000	2.0000	41.5189	21.5800	0.0434
Greenschist	2.1000	0.6000	0.0090	152.2000	0.0253

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Lith	%CaO	%MgO	%S	Uniaxial Compressive Strength (Mpa)	Geometric Mean Hydraulic Conductivity (μm/sec)
Gypsum	1.2000	2.8000	0.2900	256.9500	0.0001
Hornblende-gneiss	NA	NA	NA	NA	NA
Hornfels	6.7900	5.3100	0.0501	128.8000	0.0083
Ice	0.0000	0.0000	0.0494	137.0800	0.0001
Igneous	3.1000	1.3000	0.0500	136.5000	0.0338
Lamprophyre	0.0000	0.0000	0.0500	102.9500	0.0244
Latite	33.2000	2.7000	0.1000	108.6200	0.1480
Limestone	25.2500	6.1400	0.6500	30.9000	0.0028
Marble	27.6000	1.6000	0.6800	85.9200	0.0343
Marl	7.7000	4.0000	0.0030	101.3000	1.4000
Marlstone	8.7000	5.7000	0.0154	101.3000	1.4000
Metaandesite	2.2000	0.5000	0.0025	101.3000	1.4000
Metabasalt	8.9000	5.8000	0.0270	131.7000	0.8000
Metadacite	9.5000	6.8000	0.0060	131.7000	0.8000
Metadiabase	1.6000	0.7000	0.0070	131.7000	0.8000
Metagabbro	1.9000	1.4000	0.0005	117.3000	0.0122
Metagranite	4.4700	3.4800	0.0468	151.9600	2.5900
Metagraywacke	1.0000	0.2000	0.0040	101.3000	1.4000
Metamorphic	1.7000	2.3000	0.0900	68.8000	0.2500
Metarhyolite	2.2000	1.1000	0.0045	172.1700	0.0473
Mica-schist	1.7000	0.5000	0.0192	155.7900	0.0004
Migmatite	1.7000	0.5000	0.0192	155.7900	0.0004

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Lith	%CaO	%MgO	%S	Uniaxial Compressive Strength (Mpa)	Geometric Mean Hydraulic Conductivity (µm/sec)
Monzogranite	2.3000	0.9000	0.1850	174.6000	0.0000
Monzogranite	1.1000	0.4000	0.0030	152.2000	0.0253
Monzonite	0.3000	3.5000	0.0300	68.8000	0.2500
Muscovite-gneiss	NA	NA	NA	146.0000	50.0000
Muscovite-schist	2.1000	1.5000	0.0100	152.2100	0.0300
Mylonite	2.5000	2.7000	0.2880	152.2100	0.0300
Orthogneiss	7.0600	1.5400	3.1333	0.0300	0.0444
Paragneiss	0.8000	0.2000	0.0500	140.8800	0.0000
Peat	3.5000	32.5000	0.0900	125.7300	0.0398
Pegmatite	2.7000	0.8000	0.0144	98.0000	0.0110
Peridotite	0.7000	2.6000	0.0450	75.0300	0.0233
Phonolite	7.8000	13.3000	0.0300	76.0000	0.1350
Phyllite	14.6000	17.0000	0.0900	130.7100	2.0500
Picrite	6.8000	3.6000	0.0365	181.8700	0.0000
Pyroxenite	1.3000	2.9000	0.0600	68.8000	0.2500
Quartz-diorite	0.6000	1.1000	0.0100	242.4300	0.0343
Quartz-feldspar-schist	1.1000	0.4000	0.0200	203.7300	2.2400
Quartzite	7.7000	3.3000	22.4600	22.3100	0.0001
Rhyolite	1.4700	1.1100	0.0200	0.9600	16.0000
Salt	1.5000	1.0000	0.0300	88.0600	0.4510
Sand	NA	NA	0.5643	68.6000	0.0063
Sandstone	9.7000	0.7300	0.1880	65.6600	0.0001

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Lith	%CaO	%MgO	%S	Uniaxial Compressive Strength (Mpa)	Geometric Mean Hydraulic Conductivity (µm/sec)
Sedimentary	0.3000	35.1000	0.1700	86.5900	0.0297
Sedimentary-breccia	3.0900	1.4300	0.0700	0.3800	0.0066
Serpentinite	0.9900	1.7000	0.1300	62.4500	0.0016
Silt	27.6000	2.3000	0.0200	202.8300	0.3160
Siltstone	0.4000	1.6000	0.1400	144.2800	0.0000
Skarn	2.1000	0.7000	0.1700	218.4200	0.0000
Slate	0.6000	0.2000	0.0110	188.6800	0.0001
Syenite	NA	NA	NA	118.2500	0.0842
Syenogranite	4.9000	2.1000	0.0100	124.3700	0.0000
Tectonite	0.0000	0.0000	0.0200	232.5500	0.0775
Tonalite	NA	NA	NA	30.3700	0.0048
Trachyte	NA	NA	NA	30.3700	0.0048
tuff	NA	NA	NA	30.3700	0.0048
tuff-Andesite	NA	NA	NA	30.3700	0.0048
tuff-Basalt	NA	NA	NA	30.3700	0.0048
tuff-Rhyolite	7.5900	5.9800	0.0265	95.0000	0.1110
tuff-Trachyte	NA	NA	NA	NA	NA
Volcanic					
Water					

Source: Olson & Hawkins, 2012

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Table A2.2 Sources of environmental data

Properties	Sources	Type	Extent	Resolution	Year
Geology	Institute for Geosciences and Natural Resources (BGR) [2]	Vector	Germany	1:200,000	2007
Climate	DWD Climate Data Center [3]	Raster	Germany	1km x 1km	1981-2015
Available water capacity	Institute for Geosciences and Natural Resources (BGR) [4]	Raster	Germany	250m x 250m	2015
Bulk density	European Soil Data Centre (ESDAC) [5]	Raster	Europe	1km x 1km	2013
Organic matter content	European Soil Data Centre (ESDAC) [6]	Raster	Europe	1km x 1km	2015
Soil erodibility	European Soil Data Centre (ESDAC) [7]	Raster	Europe	500m x 500m	2014
Soil permeability	Institute for Geosciences and Natural Resources (BGR) [8]	Vector	Europe	1:200,000	2016
Soil depth	Institute for Geosciences and Natural Resources (BGR) [9]	Raster	Germany	250m x 250m	2015
Water table depth	Fan, Y., H. Li, G. Miguez Macho (2013) [10]	Raster	World	1km x 1km	2013
Enhanced vegetation index (EVI)	MODIS Vegetation Indices [11]	Raster	Germany	1km x 1km	2005-2016
Groundwater recharge veolcity	Institute for Geosciences and Natural Resources (BGR) [4]	Raster	Germany	1km x 1km	2008

Table A2.3 Highly correlated variables

Var 1	Var 2	Correlation coeffficient (EC model)	Correlation coeffficient (Ca^{2+}/Mg^{2+}) model)	Correlation coefficient (SO_4^{2-})
Mean catchment elevation	Mean compressive strength	0.76	0.70	0.75
Mean catchment elevation	Mean annual precipitation	0.75	0.71	0.73
Mean catchment elevation	Mean freeze days	0.89	0.87	0.86
Mean catchment elevation	Mean water table depth	0.79	0.77	0.78
Mean catchment elevation	Mean recharge speed	0.72	0.70	0.72
Mean annual precipitation	Mean recharge speed	0.89	0.91	0.91
Mean catchment elevation	Mean annual temperature	-0.95	-0.94	-0.94
Mean compressive strength	Mean annual temperature	-0.72		-0.71
Mean freeze days	Mean annual temperature	-0.94	-0.94	-0.94
Mean water table depth	Mean annual temperature	-0.74	-0.74	-0.72

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Table A2.4 Values of α and λ values of optimal models in elastic net regression

Models	Alpha (α)	Lamda (λ)
EC	1	0.0001
Ca^{2+}	0	0.0405
Mg^{2+}	1	0.0102
SO_4^{2-}	0.78	0.0001

Table A2.5 The size of tree (mtry) and out of bag (OOB) errors for RF models

Models	mtry	OOB error
EC	3	0.028
Ca^{2+}	3	0.053
Mg^{2+}	4	0.042
SO_4^{2-}	6	0.036

Table A2.6 Coefficient and order of important variables in LR and RF models

Random forest model				**Linear Regression model with elastic net**			
Predictor	**Direction**	**% IncMSE**	**Rank**	**Predictor**	**Direction**	**Coefficient**	**Rank**
Electrical Conductivity (log EC)							
				Intercept	+	3.69E+00	
Mean annual precipitation	-	61	1	Percent CaO	+	3.05E-01	1
Percent CaO	+	39	2	Mean annual temperature	+	9.15E-02	2

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Random forest model				Linear Regression model with elastic net			
Predictor	**Direction**	**% IncMSE**	**Rank**	**Predictor**	**Direction**	**Coefficient**	**Rank**
Percent S	+	39	3	Percent S	+	6.88E-02	3
Mean annual temperature	+	38	4	Mean annual precipitation	-	-1.57E-02	4
Mean soil depth	+	36	5	Percent MgO	+	1.70E-02	5
Mean EVI	+	35	6	Log catchment area	+	1.34E-02	6
Soil mean organic matter content	+	34	7	Mean EVI	+	5.87E-03	7
Mean hydraulic conductivity	+	30	8	Mean hydraulic conductivity	+	2.67E-03	8
				Mean soil depth	+	4.86E-04	9
				Mean water table depth	-	-1.19E-03	10
				Mean catchment elevation	-	-1.09E-03	11
				Mean compressive strength	-	-6.39E-04	12

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Random forest model				Linear Regression model with elastic net			
Predictor	**Direction**	**% IncMSE**	**Rank**	**Predictor**	**Direction**	**Coefficient**	**Rank**
				Mean soil permeability	-	-2.69E-04	13
				Mean available water capacity	-	-1.59E-04	14
				Mean recharge speed	-	-1.46E-04	15
Calcium (log Ca^{2+})							
				Intercept	+	1.68E+00	
Mean annual precipitation	-	55	1	Percent CaO	+	1.64E+00	1
Percent CaO	+	44	2	Mean annual temperature	+	6.66E-01	2
Mean EVI	+	42	3	Percent S	+	1.50E-01	3
Percent S	+	41	4	Mean annual precipitation	-	-6.55E-02	4
Mean hydraulic conductivity	+	36	5	Log soil mean organic matter content	+	5.18E-02	5

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Random forest model				Linear Regression model with elastic net			
Predictor	**Direction**	**% IncMSE**	**Rank**	**Predictor**	**Direction**	**Coefficient**	**Rank**
Mean compressive strength	+	36	6	Mean hydraulic conductivity	+	3.78E-02	6
Mean annual temperature	+	30	7	Mean soil erodibility	+	1.87E-02	7
				Percent MgO	+	1.08E-02	8
				Mean soil permeability	-	-1.17E-02	9
				Log catchment area	+	1.03E-02	10
				Mean soil depth	+	9.54E-03	11
				Mean water table depth	-	-2.39E-03	12
				Mean EVI	+	5.33E-03	13
				Mean freeze days	-	-2.16E-03	14
				Mean available water capacity	-	-9.04E-04	15
				Mean recharge	-	-6.92E-04	16

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Random forest model				Linear Regression model with elastic net			
Predictor	Direction	% IncMSE	Rank	Predictor	Direction	Coefficient	Rank
				speed			
				Mean compressive strength	-	-5.24E-04	17
				Mean bulk density	+	3.53E-04	18
				Mean catchment elevation	-	-3.67E-04	19
Magnesium (log Mg^{2+})							
				Intercept	+	1.24E+00	1
Mean annual precipitation	+	59	1	Percent S	-	1.69E-01	2
Percent MgO	+	47	2	Percent MgO	+	1.36E-01	3
Percent S	+	36	3	Mean EVI	+	8.23E-02	4
Mean annual temperature	+	35	4	Annual precipitation	-	-2.32E-02	5
Mean EVI	+	32	5	Annual freezed days	-	-5.35E-03	6
Mean compressive strength	-	38	6	Mean soil permeability	-	-4.25E-03	7

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Random forest model				Linear Regression model with elastic net			
Predictor	Direction	% IncMSE	Rank	Predictor	Direction	Coefficient	Rank
Mean soil erodibility	+	27	7	Log catchment area	-	-2.52E-03	8
Mean soil permeability	-	20	8	Mean recharge speed	-	-8.19E-04	9
				Mean bulk density	+	5.45E-04	10
				Mean compressive strength	-	-3.39E-04	11
				Mean available water capacity	-	-9.85E-05	12
				Mean catchment elevation	-	-5.11E-05	13
Sulphate (log SO_4^{2-})							
				Intercept	+	2.00E+00	
Mean annual precipitation	-	98	1	Percent CaO	+	1.28E+00	1
Mean soil permeability	-	37	2	Mean annual temperature	+	5.86E-01	2

Appendix A2

Random forest model				Linear Regression model with elastic net			
Predictor	**Direction**	**% IncMSE**	**Rank**	**Predictor**	**Direction**	**Coefficient**	**Rank**
Mean hydraulic conductivity	+	30	3	Percent S	+	1.17E-01	3
Mean soil depth	+	28	4	Log soil mean organic matter content	+	6.04E-02	4
Mean available water capacity	+	27	5	Mean bulk density	+	5.57E-02	5
Mean annual temperature	+	25	6	Percent MgO	+	3.13E-02	6
Percent S	+	24	7	Mean EVI	+	2.35E-02	7
Mean bulk density	+	24	8	Mean hydraulic conductivity	+	1.40E-02	8
Mean EVI	+	20	9	Mean soil permeability	-	-1.23E-02	9
Percent MgO	+	12	10	Annual freezed days	-	-1.04E-02	10
				Log catchment area	-	-5.19E-03	11

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Random forest model				Linear Regression model with elastic net			
Predictor	**Direction**	**% IncMSE**	**Rank**	**Predictor**	**Direction**	**Coefficient**	**Rank**
				Mean water table depth	+	1.33E-03	12
				Mean catchment elevation	-	-1.15E-03	13
				Mean soil erodibility	+	8.07E-04	14
				Mean annual precipitation	-	-6.16E-04	15
				Mean soil depth	+	2.82E-04	16
				Mean compressive strength	-	-2.22E-04	17
				Mean available water capacity	+	2.11E-04	18
				Mean recharge speed	-	-1.05E-04	19

Table A2.7 Change in temperature and precipitation for Germany in period from 2070-2100

Period	Temperature (^{0}C)			Precipitation (mm/year)		
	Min	**Mean**	**Max**	**Min**	**Mean**	**Max**
Current (1981-2015)	-3.62	8.93	11.38	399.00	807.02	3258.47
RCP2.6 (2070-2100)	-0.52	10.92	13.83	421.00	698.77	1785.00
RCP8.5 (2070-2100)	2.62	13.64	16.49	418.00	691.43	1740.00

Appendix A2

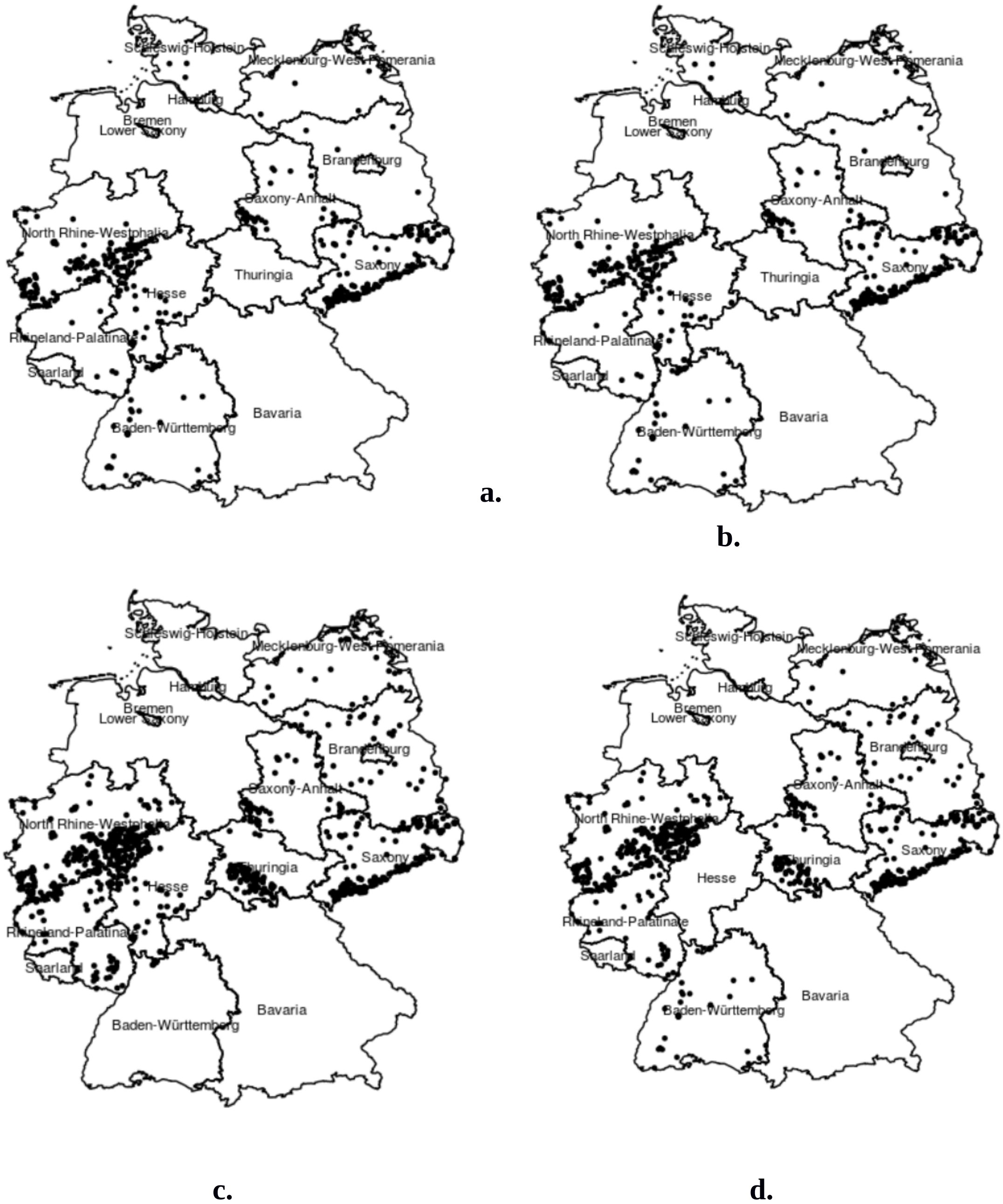
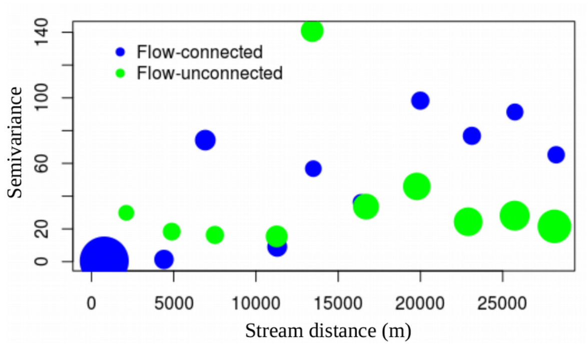
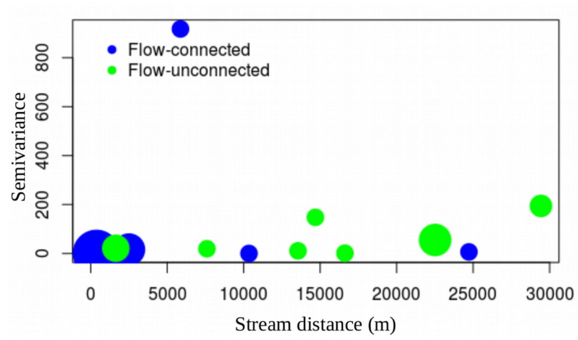


Fig A2.1 Location of monitoring sites a) Ca sites (266 sites) b) Mg sites (266 sites) c) EC sites (410 sites) d) SO_4^{2-} sites (357 sites)



a.



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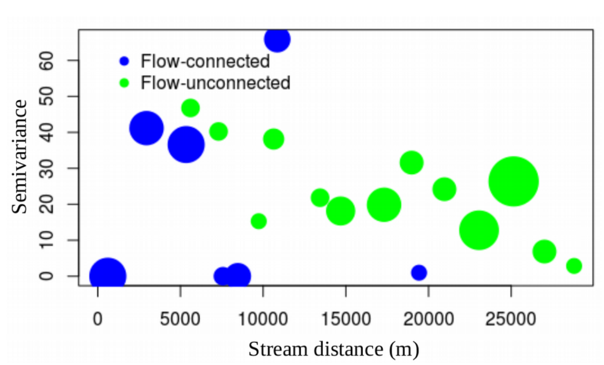

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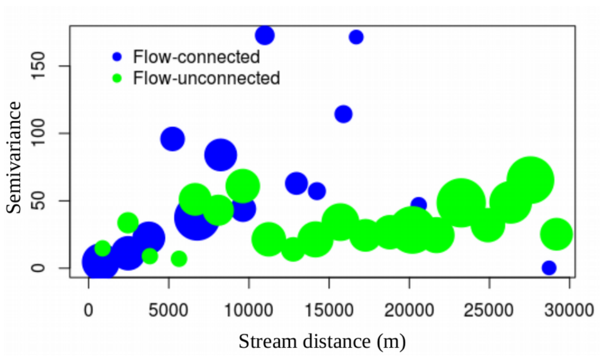

d.

Fig A2.2 Semivariogram of EC measurements in the German states a) Saxony b) Hesse c)North Rhine-Westphalia d)Thuringia

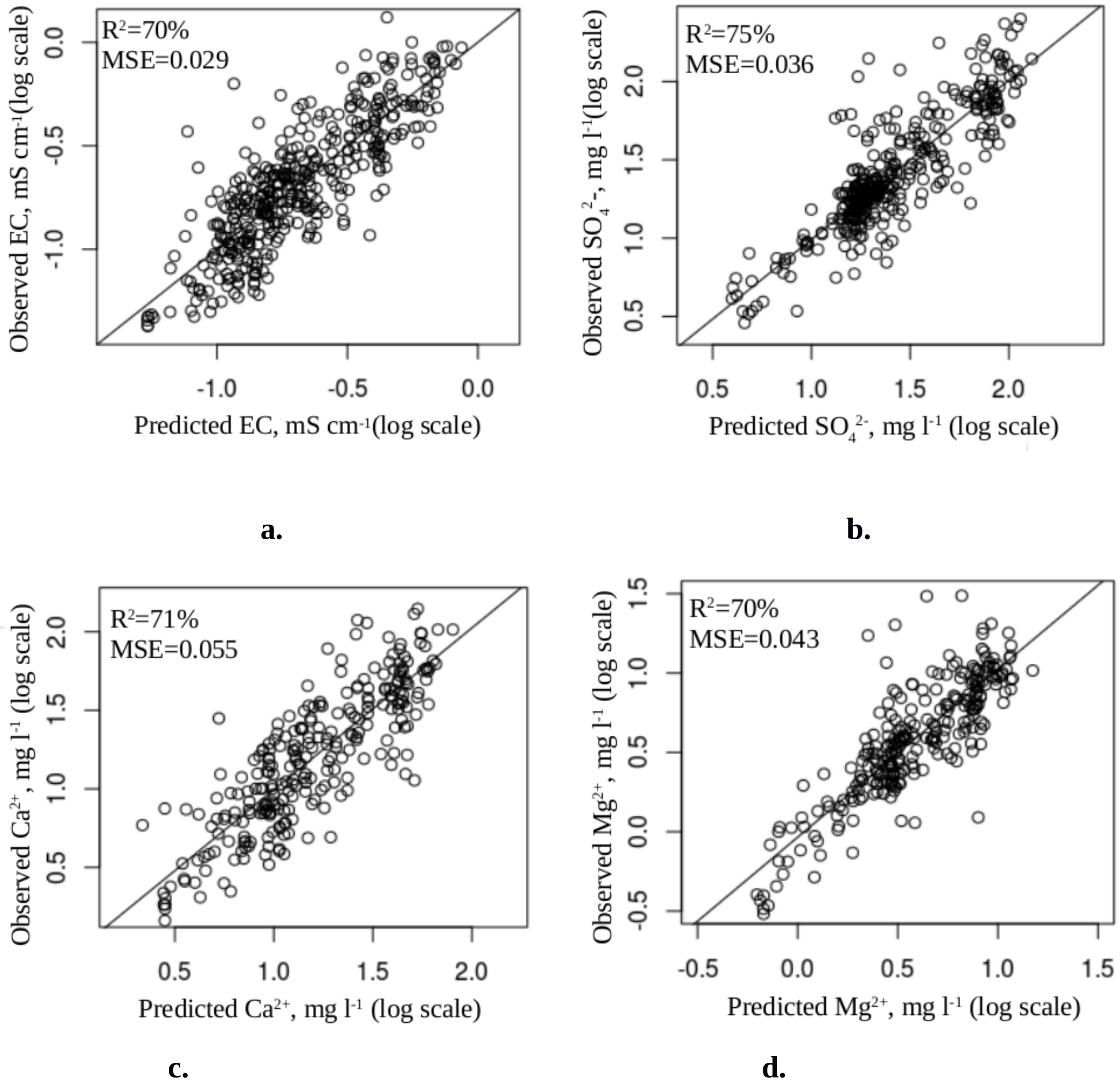

Fig A.2.3 Plots of predicted versus observed values in LR a) Electrical conductivity b) Sulfate c) Calcium d) Magnesium

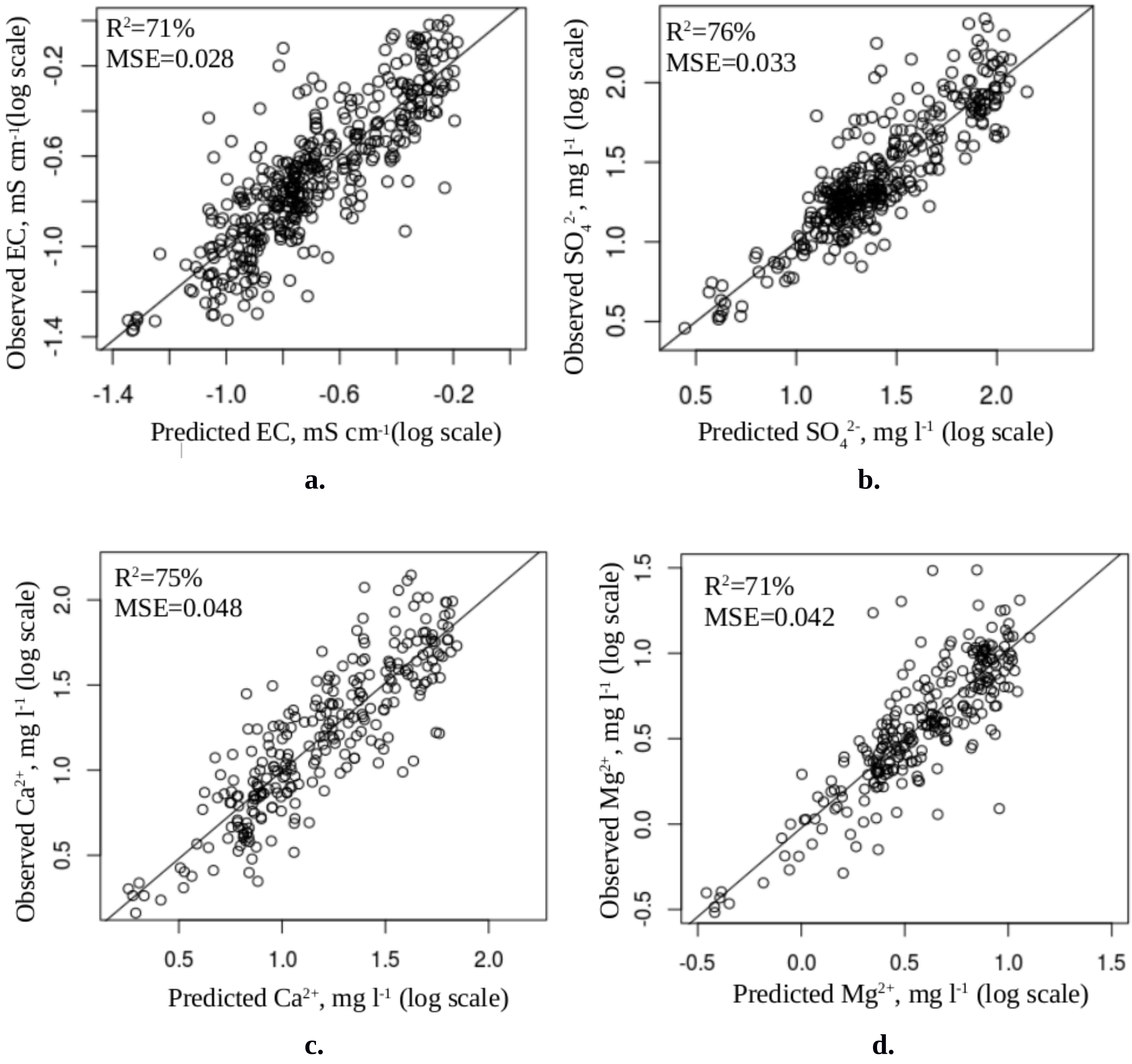

Fig A2.4 Plots of predicted versus observed values in RF a) Electrical conductivity b) Sulfate c) Calcium d) Magnesium

Appendix A2

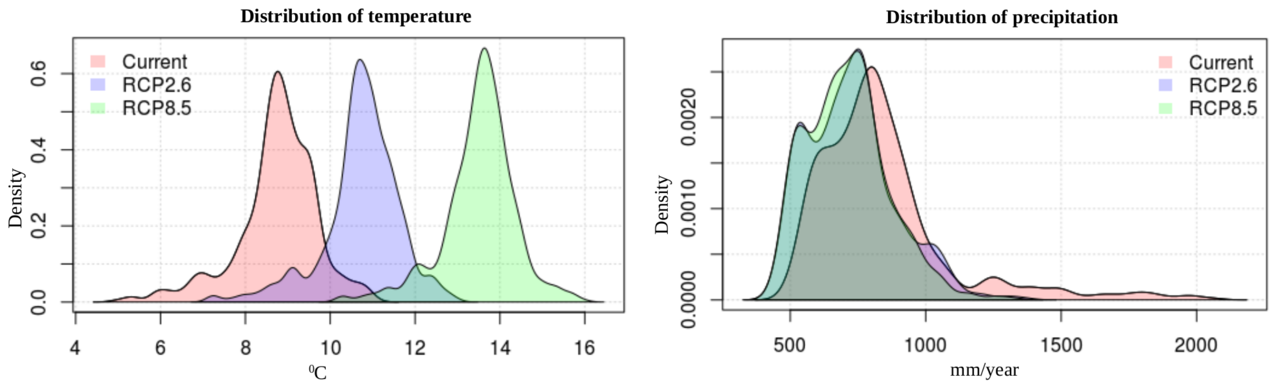

Fig A2.5. Distribution of temperature and precipitation in 610 sample sites in Germany in period from 2070-2100 a) Temperature b) Precipitation

Reference

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APPENDIX A3

Table A3.1: Sources of datasets. For details see Le et al. 2019

Datasets	Variables	Shortcut	Sources	Unit
EC	Electrical conductivity	EC	German federal state authorities	mS cm^{-1}
Nutrients	Phosphate	PO_4^{2-}		mg L^{-1}
	Nitrate	NO_3^-		mg L^{-1}
Geology	Catchment mean CaO	CaO	Institute for Geosciences and Natural Resources (BGR)	%
	Catchment mean S	S		%
	Catchment hydraulic conductivity	HC		10^{-6} m s^{-1}
Climate	Precipitation	Precip	DWD Climate Data Center	mm/$year^{-1}$
	Air temperature	Temp		°C
Soil	Organic matter content	OC	European Soil Data Centre (ESDAC)	%
	Soil depth	-	Institute for Geosciences and Natural Resources (BGR)	m
Vegetation	Enhanced vegetation index (EVI)	EVI	MODIS Vegetation Indices	Dimensionless

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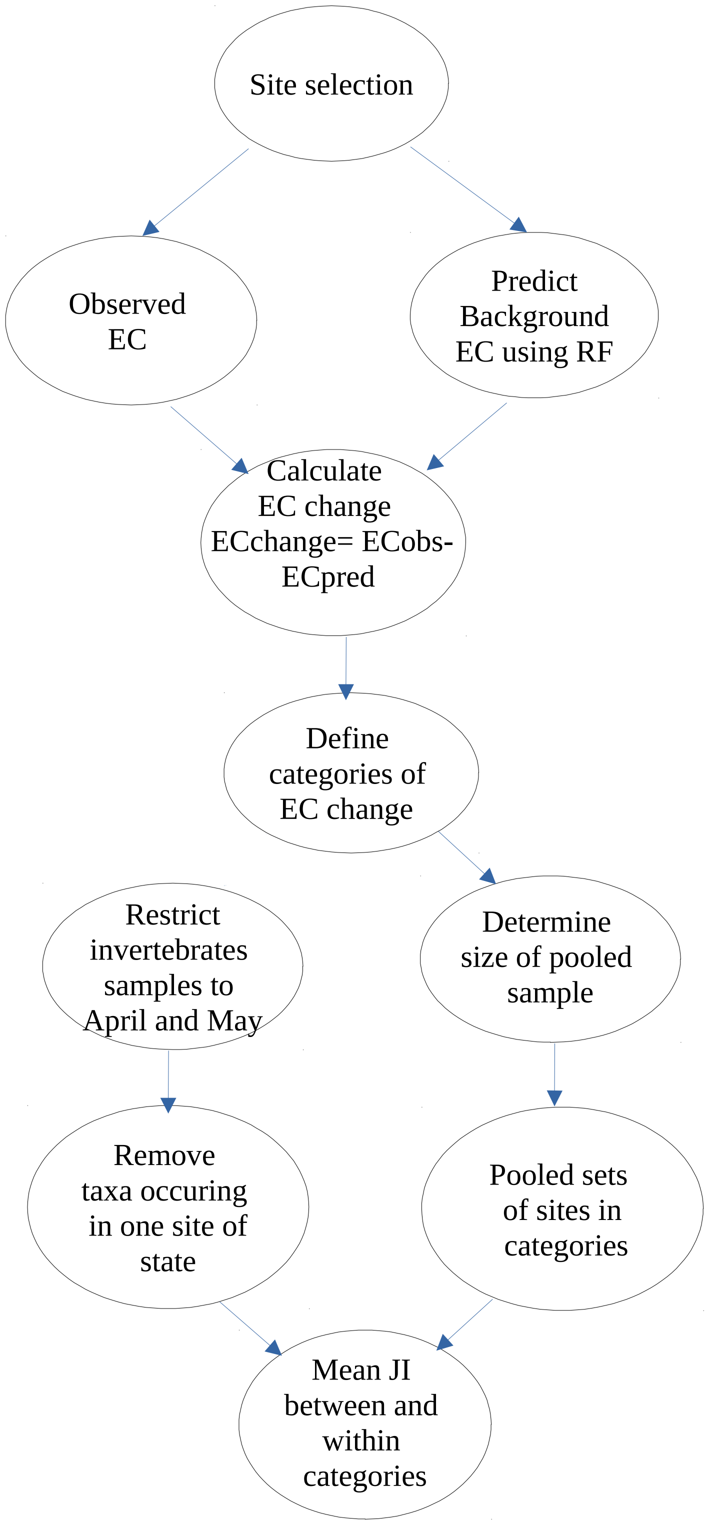

Site selection
Observed EC
Predict Background EC using RF
Calculate EC change ECchange= ECobs-ECpred
Define categories of EC change
Restrict invertebrates samples to April and May
Determine size of pooled sample
Remove taxa occuring in one site of state
Pooled sets of sites in categories
Mean JI between and within categories

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Fig A3.1: Workflow of taxa turnover analysis with EC: electrical conductivity; RF: random forest; ECobs: observed electrical conductivity; ECpred: electrical conductivity predicted by using random forest model; and JI: Jaccard index

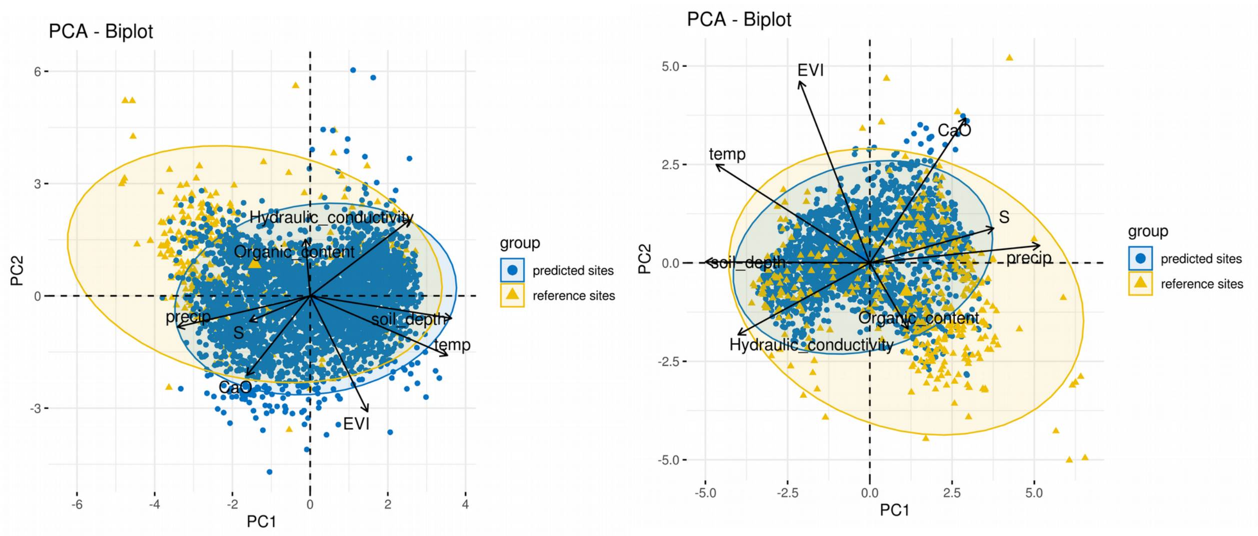

Fig A3.2: Distribution of sampling sites and sites used in model training (reference sites) with respect to the space of environmental variables

a) several sampling sites are outside of environmental space of the reference sites

b) all sampling sites are inside of environmental space of the reference sites.

Abbreviations: precipitation (precip), air temperature (temp), % S (S) and % CaO (CaO) in bedrock, and enhanced vegetation index (EVI)

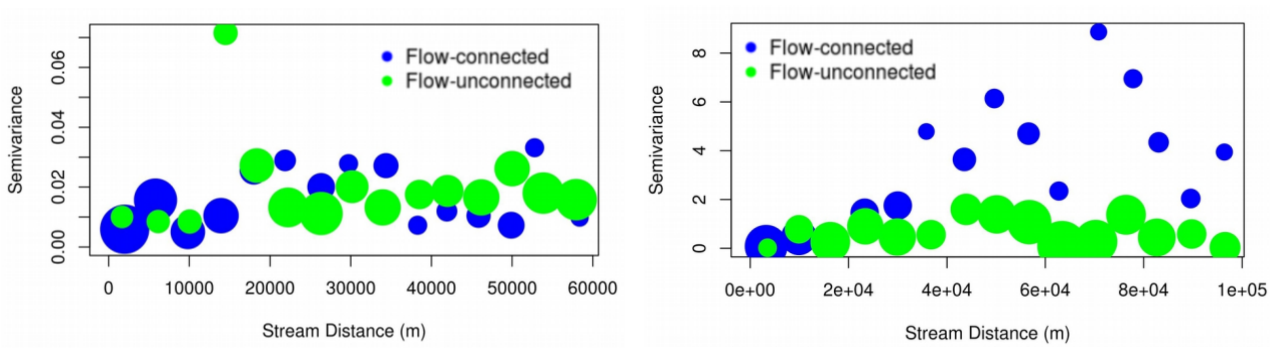

Fig A3.3: Semivariogram of EC changes in a. North Rhine-Westphalia b. Thuringia

Table A3.2: Number of samples and pooled sample sets in observed EC categories in North Rhine-Westphalia and Thuringia.

Observed EC category	**Number of sites**	**Number of pooled sets**	**Total no of sites randomly included in pooled sample sets**	**Range of observed EC [ms/S]**
		North Rhine-Westphalia		
1	21	4	20	[0.1 – 0.2)[a]
2	30	6	30	[0.2 - 0.3)
3	24	4	20	[0.3 - 0.4)
4	7	1	5	[0.4 - 0.5)
5	20	4	20	[0.5 - 0.7)[b]
6	36	7	35	>=0.7[c]
Total	138	20	120	
		Thuringia		
1	28	5	25	[0.05 - 0.2)[d]
2	13	2	10	[0.2 - 0.3)
3	17	3	15	[0.3 - 0.4)
4	11	2	10	[0.4 - 0.5)
5	15	3	15	[0.5 - 0.7)
6	17	3	15	[0.7 - 1.0)
7	20	4	20	>=1.0
Total	121	22	110	

Super sample size: 5 samples

[a]No site with EC < 0.1 mS cm^{-1}

[b]2 sites with EC in range [0.5-0.6)

[c] 4 sites with EC >1 mS cm^{-1} .

[d]4 sites with EC in range [0.05-0.1)

Categories with a low number of sites were combined to achieve a relatively homogeneous distribution across EC categories.

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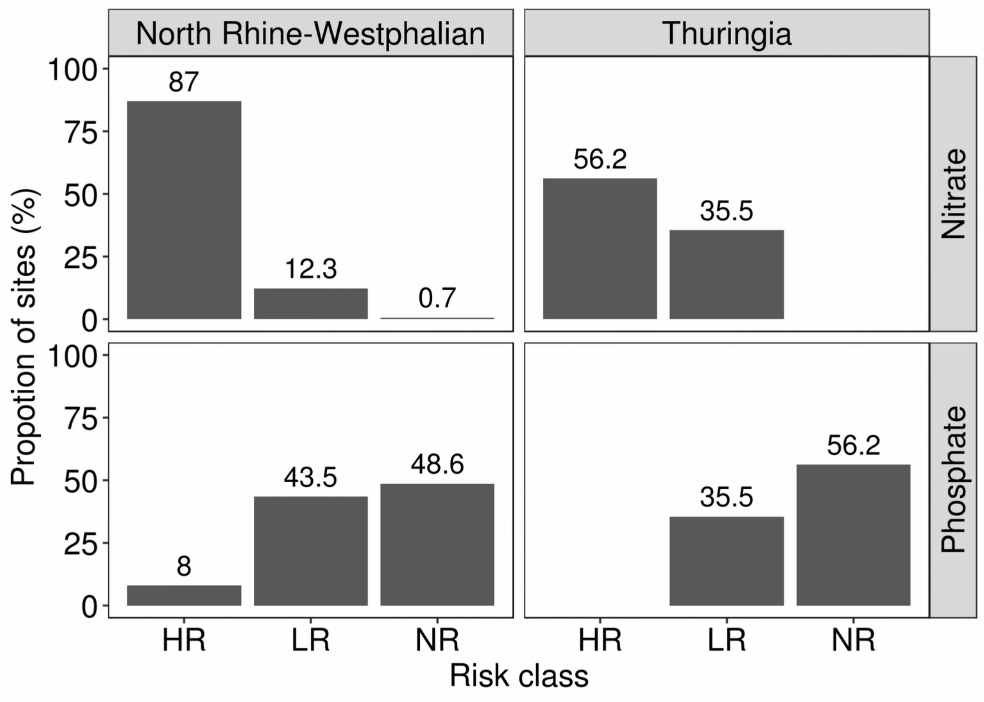
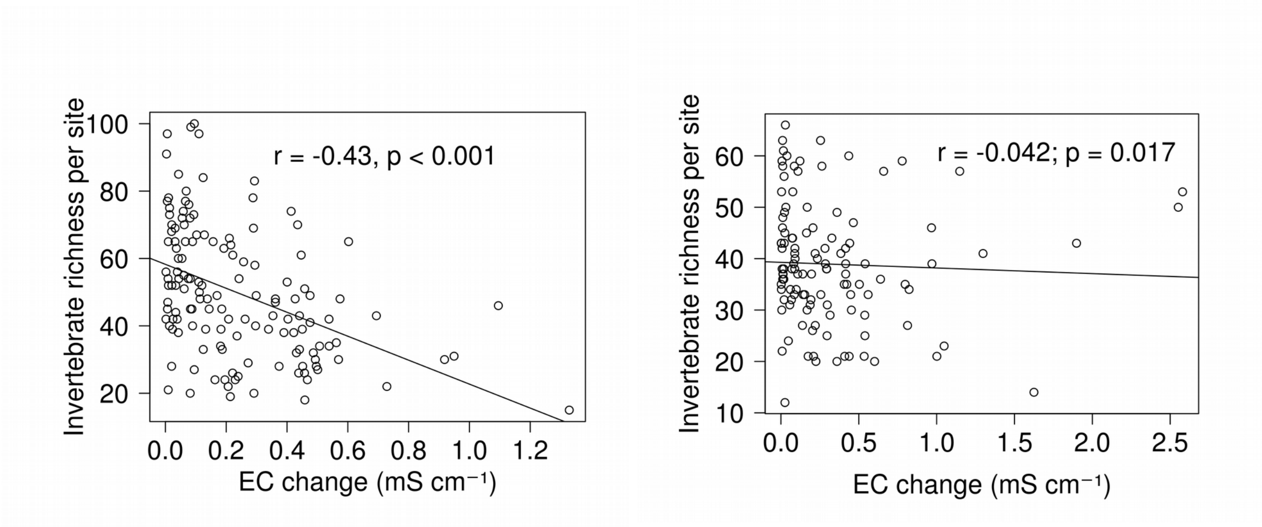
Fig A3.4: Proportion of sites exceeding low risk (LR) and high-risk (HR) thresholds defined for two states. See section 3.3 for details on the thresholds. NR, negligible risk.





a. **b.**

Fig A3.5: Invertebrate richness per site against EC change in a. North Rhine-Westphalia & b. Thuringia**,** lines give the linear model (richness predicted by EC change)

Land use along gradient of EC change categories

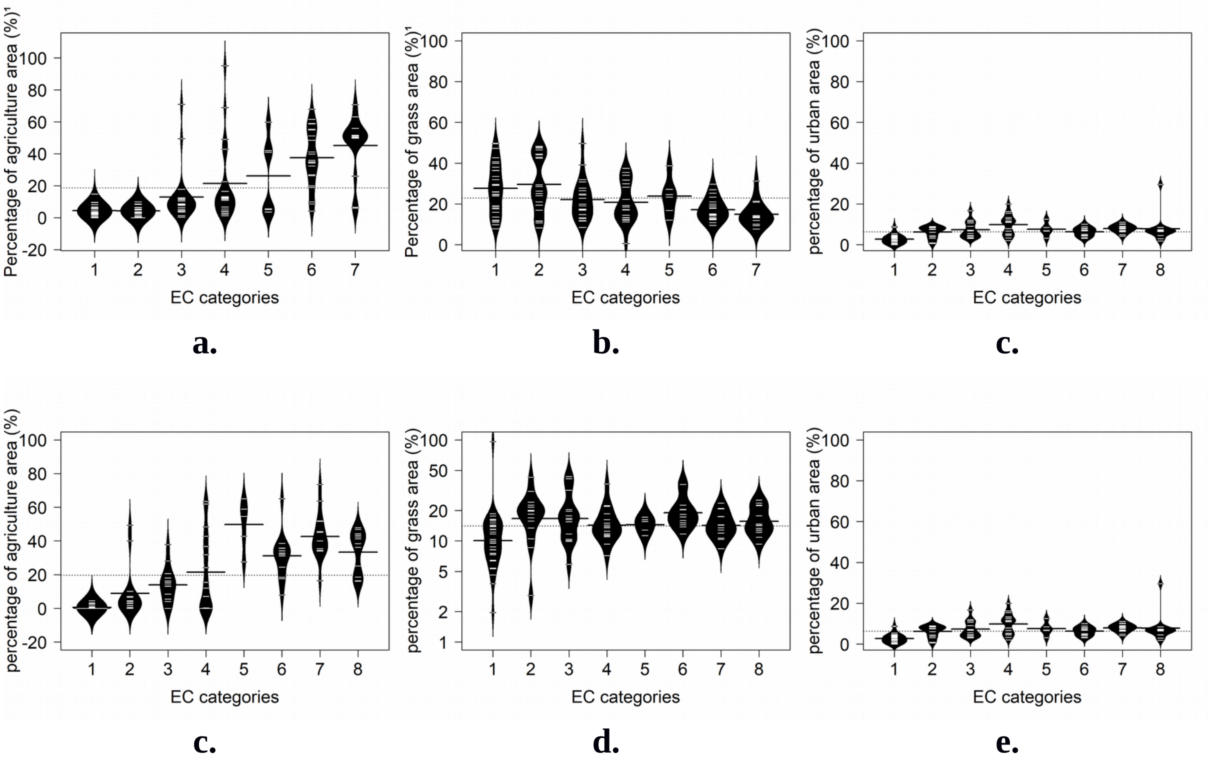


Fig A3.6: Agricultural, grass and urban land use across EC change categories in North Rhine-Westphalia (a, b, c); Thuringia (d, e, f). White lines represent observations, where the length of the line indicates the relative frequency.

The percentage of the agriculture and urban areas increased more or less constantly with increasing category of EC change in both states North Rhine-Westphalia and Thuringia (Fig. S4), whereas percentage of the grass area decreased with increasing of EC change. Stream EC change and percentage of the agriculture area showed the medium positive correlations in two states (North Rhine-Westphalia: $r = 0.67$, $p < 0.001$; Thuringia: $r = 0.4$, $p < 0.001$), whereas it was rather weak for urban (North Rhine-Westphalia: $r = 0.29$, $p < 0.001$; Thuringia: $r = 0.13$, $p < 0.001$). On the contrary, a weak negative correlation between EC change and percentage of the grass area was observed in both states (North Rhine-Westphalia: $r = -0.37$, $p < 0.001$; Thuringia; $r = -0.016$, $p = 0.28$). The relationship between EC and land use is expected due to erosion, irrigating, fertilizers and grazing, and wastewater effluents that would drive freshwater conductivity (Hoagstrom. C, 2009; J. Causape et al., 2004, Piscart et al., 2005; Dikio, 2010; Müller and Gächter 2011, Kaushal et al., 2008) and then contributed to the decrease in invertebrate richness.

Table A3.3: The mean Jaccard's index of similarity between observed EC categories North Rhine-Westphalia.

Observed EC categories	Range of observed EC	1	2	3	4	5	6
		[0.1- 0.2)	[0.2 - 0.3)	[0.3 - 0.4)	[0.4 - 0.5)	[0.5 - 0.7)	>=0.7
1	[0.1- 0.2)	0.53					
2	[0.2 - 0.3)	0.51 (-0.09)	0.48				
3	[0.3 - 0.4)	0.48 (0.57)	0.50 (0.01)	0.51			
4	[0.4 - 0.5)	0.50 (0.5)	0.51 (-0.27)	0.52 (0)	NA		
5	[0.5 - 0.7)	0.36 (0.40[a])	0.38 (0.35[a])	0.39 (0.24)	0.42 (-0.67)	0.33 (0.01)	
6	>=0.7	0.23 (0.49[a])	0.25 (0.52[a])	0.27(0.26)	0.26 (0.007)	0.28	0.28

Global ANOSIM: 0.19, p=0.025
In bracket: pairwise ANOSIM statistic R
[a] pairwise ANOSIM p value < 0.05
NA: undefined (a result of only one pooled sample set)

Table A3.4: The mean Jaccard's index of similarity between observed EC categories Thuringia.

Observed EC categories	Range of Observed EC	1	2	3	4	5	6	7
		[0.05 - 0.2)	[0.2 - 0.3)	[0.3 - 0.4)	[0.4 - 0.5)	[0.5 - 0.7)	[0.7 - 1.0)	>=1.0
1	[0.05 - 0.2)	0.48						
2	[0.2 - 0.3)	0.48 (0.05)	0.45					
3	[0.3 - 0.4)	0.43 (0.50)	0.43 (0.25)	0.45				
4	[0.4 - 0.5)	0.41 (0.6)	0.42 (0.00)	0.47 (-0.33)	0.39			
5	[0.5 - 0.7)	0.32 (0.97[a])	0.34 (0.83)	0.40 (0.33)	0.44 (-0.42)	0.44		
6	[0.7 - 1.0)	0.31 (1.00[a])	0.34 (1.00)	0.37 (0.74)	0.46 (0.25)	0.44 (0.22)	0.50	
7	>=1.0	0.26 (1.00[a])	0.27 (1.00)	0.30 (1.00[a])	0.37 (0.78)	0.39 (0.41)	0.41 (0.48)	0.43

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Global ANOSIM R=0.60, p=0.001

In bracket: pairwise ANOSIM statistic R

[a] pairwise ANOSIM p value < 0.05

NA: undefined (a result of only one pooled set)

Table A3.5: Results from the Indicator Taxa Analysis (IndVal) for EC change categories for North Rhine-Westphalia.

EC change gradient categories	Class	Order	Taxon	A[a]	B[b]	IndVal	p-value
1	Insecta	Ephemeroptera	*Ecdyonurus venosus*-Gr.	0.36	0.88	0.56	0.001
1	Insecta	Plecoptera	*Isoperla sp.*	0.41	0.75	0.55	0.002
1	Insecta	Trichoptera	*Anomalopterygella chauviniana*	0.36	0.81	0.54	0.006
1	Insecta	Plecoptera	*Siphonoperla sp.*	0.34	0.75	0.50	0.005
1	Insecta	Diptera	*Ibisia marginata*	0.67	0.38	0.50	0.002
1	Insecta	Ephemeroptera	*Habroleptoides confusa*	0.32	0.75	0.49	0.005
1	Insecta	Plecoptera	*Protonemura sp.*	0.37	0.53	0.44	0.04
1	Insecta	Trichoptera	*Lepidostoma hirtum*	0.28	0.69	0.44	0.034
1	Insecta	Plecoptera	*Perla marginata*	0.56	0.34	0.44	0.017
1	Insecta	Plecoptera	*Amphinemura sp.*	0.34	0.53	0.43	0.040
1	Insecta	Trichoptera	*Micrasema longulum*	0.44	0.41	0.42	0.017
1	Insecta	Plecoptera	*Brachyptera risi*	0.39	0.44	0.41	0.029
1	Insecta	Coleoptera	*Limnius opacus*	1.00	0.16	0.40	0.015
1	Insecta	Trichoptera	*Polycentropus flavomaculatus ssp.*	1.00	0.13	0.35	0.022

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EC change gradient categories	Class	Order	Taxon	A[a]	B[b]	IndVal	p-value
1	Insecta	Trichoptera	*Oecetis testacea*	0.43	0.28	0.35	0.043
2	Insecta	Ephemeroptera	*Epeorus assimilis*	0.38	0.73	0.53	0.003
2	Insecta	Ephemeroptera	*Rhithrogena semicolorata*-Gr	0.28	0.91	0.50	0.001
2	Insecta	Coleoptera	*Hydraena gracilis*	0.28	0.86	0.49	0.011
2	Insecta	Ephemeroptera	*Habrophlebia lauta*	0.39	0.55	0.46	0.024
2	Insecta	Ephemeroptera	*Torleya major*	0.33	0.64	0.46	0.017
2	Insecta	Plecoptera	*Leuctra sp.*	0.29	0.73	0.45	0.038
2	Insecta	Ephemeroptera	*Ephemera danica*	0.26	0.77	0.45	0.027
2	Insecta	Coleoptera	*Orectochilus villosus*	0.32	0.64	0.45	0.026
2	Insecta	Trichoptera	*Glossosoma*	0.53	0.32	0.41	0.019
2	Insecta	Trichoptera	*Polycentropus flavomaculatus flavomaculatus*	0.28	0.59	0.41	0.046
2	Clitellaa	Oligochaeta	*Nais elinguis*	0.61	0.27	0.41	0.023
2	Insecta	Trichoptera	*Chaetopterygini*	0.81	0.14	0.33	0.031
3	Insecta	Diptera	*Diamesa insignipes*	0.86	0.19	0.40	0.013
3	Insecta	Trichoptera	*Allogamus auricollis*	0.38	0.43	0.40	0.044
3	Clitellaa	Oligochaeta	*Nais sp.*	1.00	0.14	0.36	0.015
3	Insecta	Hymenoptera	*Agriotypus armatus*	1.00	0.14	0.38	0.010

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EC change gradient categories	Class	Order	Taxon	A[a]	B[b]	IndVal	p-value
3	Insecta	*Ephemeroptera*	*Ecdyonurus venosus*	0.82	0.14	0.34	0.021
5	Insecta	Coleoptera	*Elmis aenea*	0.27	0.83	0.47	0.011
5	Insecta	Diptera	*Dicranota*	0.21	1	0.46	0.002
5	Insecta	Ephemeroptera	*Baetis rhodani*	0.17	1	0.42	0.017
5	Insecta	Trichoptera	*Drusinae Gen. sp.*	0.84	0.17	0.37	0.035
5	Insecta	Trichoptera	*Hydropsyche fulvipes*	0.84	0.17	0.37	0.038
6	Insecta	Trichoptera	*Hydropsyche contubernalis*	1.00	0.18	0.42	0.019
6	Insecta	Trichoptera	*Ceraclea alboguttata*	1.00	0.14	0.37	0.022
6	Bivalvia	Sphaeriida	*Sphaeriidae Gen. sp.*	1.00	0.14	0.37	0.018
6	Insecta	Trichoptera	*Mystacides nigra*	0.60	0.18	0.33	0.045
7	Malacostraca	Amphipoda	*Dikerogammarus villosus*	0.92	0.5	0.68	0.001
7	Malacostraca	Crustacea	*Limnomysis benedeni*	1.00	0.43	0.65	0.001
7	Malacostraca	Amphipoda	*Chelicorophium curvispinum*	0.82	0.43	0.59	0.001
7	Insecta	Ephemeroptera	*Ephemera glaucops*	1.00	0.29	0.53	0.002
7	Malacostraca	Isopoda	*Jaera sarsi*	1.00	0.28	0.53	0.001
7	Insect	Odonata	*Calopteryx splendens*	0.61	0.5	0.55	0.002
7	Polychaeta	Terebellida	*Hypania invalida*	0.86	0.28	0.50	0.008

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EC change gradient categories	Class	Order	Taxon	A[a]	B[b]	IndVal	p-value
7	Bivalia	Venerida	*Corbicula fluminea*	0.86	0.28	0.50	0.002
7	Gastropoda	Sorbeoconcha	*Potamopyrgus antipodarum*	0.27	0.86	0.48	0.006
7	Malacostraca	Amphipoda	*Gammarus tigrinus*	0.76	0.28	0.47	0.015
7	Insecta	Ephemeroptera	*Caenis sp.*	0.59	0.36	0.46	0.012
7	Clitellata	Oligochaeta	*Tubificidae Gen.sp.*	0.26	0.71	0.44	0.025
7	Clitellata	Oligochaeta	*Stylaria lacustris*	0.81	0.21	0.42	0.018
7	Malacostraca	Amphipoda	*Chelicorophium robustum*	0.82	0.21	0.42	0.014
7	Insecta	Ephemeroptera	*Caenis luctuosa*	0.48	0.36	0.41	0.021
7	Insecta	Odonata	*Coenagrionidae Gen.sp.*	0.45	0.36	0.40	0.021
7	Malacostraca	Decapoda	*Orconectes limosus*	0.70	0.21	0.39	0.028
7	Insecta	Odonata	*Platycnemis pennipes*	0.69	0.21	0.39	0.029
7	Insecta	Trichoptera	*Lype phaeopa*	1.00	0.14	0.38	0.014
7	Malacostraca	Amphipoda	*Dikerogammarus*	1.00	0.14	0.38	0.013
7	Insecta	Coleoptera	*Haliplus*	1.00	0.14	0.38	0.014
7	Insecta	Ephemeroptera	*Baetidae Gen.sp*	1.00	0.14	0.38	0.015
7	Clitellata	Arhynchobdellia	*Dina lineata*	1.00	0.14	0.38	0.016
7	Insecta	Trichoptera	*Leptoceridae Gen.sp*	1.00	0.14	0.38	0.012
7	Clitellata	Haplotaxida	*Potamothrix moldaviensis*	0.61	0.21	0.36	0.049

EC change gradient categories	Class	Order	Taxon	A[a]	B[b]	IndVal	p-value
7	Insecta	Diptera	*Chironomus riparius*-Agg	0.61	0.21	0.36	0.039

[a] proportion invertebrate are found in a single EC change gradient category.

[b] proportion an invertebrate occurs in all samples in that category.

Table A3.6: Results from the Indicator Taxa Analysis (IndVal) EC change gradient categories as groups in Thuringia.

EC change gradient categories	Class	Order	Taxon	A[a]	B[b]	IndVal	p.value
1	Rhabditophora	Seriata	*Polycelis felina*	1.00	0.39	0.62	0.002
1	Insecta	Coleoptera	*Limnius perrisi*	0.71	0.52	0.61	0.002
1	Insecta	Plecoptera	*Protonemura*	0.49	0.64	0.56	0.003
1	Insecta	Plecoptera	*Siphonoperla*	0.70	0.45	0.56	0.002
1	Insecta	Coleoptera	*Esolus*	0.61	0.52	0.56	0.001
1	Insecta	Diptera	*Prosimulium*	0.54	0.55	0.54	0.006
1	Insecta	Plecoptera	*Leuctra sp.*	0.28	0.97	0.52	0.001
1	Insecta	Trichoptera	*Glossosoma conformis*	0.83	0.32	0.52	0.004
1	Insecta	Diptera	*Ibisia marginata*	0.66	0.39	0.50	0.010
1	Insecta	Plecoptera	*Amphinemura sp.*	0.33	0.65079	0.46	0.008
1	Insecta	Trichoptera	*Anomalopterygella chauviniana*	0.29	0.87	0.50	0.004
1	Insecta	Ephemeroptera	*Habroleptoides confusa*	0.41	0.58	0.49	0.016

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EC change gradient categories	Class	Order	Taxon	A[a]	B[b]	IndVal	p.value
1	Insecta	Coleoptera	*Sericostoma sp.*	0.27	0.84	0.47	0.023
1	Insecta	Ephemeroptera	*Baetis alpinus*	0.79	0.26	0.45	0.010
1	Insecta	Trichoptera	*Odontocerum albicorne*	0.62	0.32	0.45	0.014
1	Insecta	Ephemeroptera	*Ephemerella mucronata*	0.35	0.55	0.44	0.025
1	Insecta	Ephemeroptera	*Epeorus assimilis*	0.35	0.52	0.43	0.024
1	Insecta	Trichoptera	*Micrasema longulum*	0.77	0.22	0.42	0.047
1	Insecta	Diptera	*Eloeophila*	1.00	0.16	0.40	0.022
1	Insecta	Ephemeroptera	*Rhithrogena*	0.49	0.32	0.40	0.028
1	Insecta	Ephemeroptera	*Heptageniidae*	0.71	0.19	0.37	0.043
2	Insecta	Plecoptera	*Isoperla sp.*	0.23	0.8	0.43	0.045
4	Insecta	Ephemeroptera	*Rhithrogena semicolorata-Gr*	0.32	0.56	0.42	0.034
4	Insecta	Trichoptera	*Chaetopteryx villosa ssp.*	0.59	0.25	0.38	0.050
5	Insecta	Ephemeroptera	*Ephemera danica*	0.41	1.00	0.64	0.002
5	Insecta	Trichoptera	*Halesus digitatus*	0.59	0.4	0.49	0.010
5	Bivalvia	Sphaeriida	*Sphaerium corneum*	0.44	0.4	0.42	0.049
5	Insecta	Diptera	*Simulium vernum*	0.86	0.2	0.41	0.032
6	Insecta	Trichoptera	*Lype sp.*	0.58	0.33	0.44	0.034
6	Insecta	Odonata	*Calopteryx sp.*	1.00	0.17	0.41	0.031
6	Insecta	Ephemeroptera	*Baetis fuscatus*	0.35	0.42	0.38	0.046
6	Insecta	Diptera	*Simulium sp. (Wilhelmia)*	0.55	0.25	0.37	0.044
7	Architaeniodloss	Gastropoda	*Bithynia*	0.78	0.27	0.45	0.014

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EC change gradient categories	Class	Order	Taxon	A[a]	B[b]	IndVal	p.value
	a		*tentaculata*				
7	Insecta	Diptera	*Simulium ornatum-Gr*	0.76	0.27	0.45	0.024
7	Malacostraca	Isopoda	*Asellus aquaticus*	0.27	0.73	0.44	0.025
8	Littorinimorpha	Gastropoda	*Potamopyrgus antipodarum*	0.55	0.61	0.58	0.003
8	Insecta	Diptera	*Chironominae Gen.sp.*	0.54	0.61	0.58	0.006
8	Malacostraca	Amphipoda	*Gammarus roeselii*	0.51	0.61	0.56	0.002
8	Insecta	Diptera	*Ceratopogonidae Gen.sp.*	0.39	0.54	0.46	0.030
8	Insecta	Trichoptera	*Anabolia nervosa*	0.34	0.61	0.46	0.008
8	Insecta	Coleoptera	*Nebrioporus depressus*	0.61	0.31	0.43	0.034
8	Insecta	Heteroptera	*Nepa cinerea*	1	0.15	0.39	0.037
8	Insecta	Coleoptera	*Haliplus fluviatilis*	1	0.15	0.39	0.046
8	Insecta	Ephemeroptera	*Baetis buceratus*	1	0.15	0.39	0.047
8	Clitellata	Oligochaeta	*Tubifex sp.*	1	0.15	1.39	0.046

[a] proportion invertebrate are found in a single EC change gradient category.

[b] proportion an invertebrate occurs in all samples in that category.

Table A3.7: Results from the Indicator Taxa Analysis (IndVal) EC categories.

Class	Order	Taxon	North Rhine-Westphalia					Thuringia				
			EC change gradient categories	A[a]	B[b]	IndVal	p value	EC change gradient categories	A[a]	B[b]	IndVal	p value
Insecta	Plecoptera	*Amphinemura sp.*	1	0.34	0.53	0.43	0.040	1	0.33	0.65	0.46	0.008
Insecta	Trichoptera	*Anomalopterygella chauviniana*	1	0.36	0.81	0.54	0.006	1	0.29	0.87	0.5	0.004
Insecta	Diptera	*Ibisia marginata*	1	0.67	0.38	0.5	0.002	1	0.66	0.39	0.51	0.010
Insecta	Ephemeroptera	*Habroleptoides confusa*	1	0.32	0.75	0.49	0.005	1	0.41	0.58	0.49	0.016
Insecta	Plecoptera	*Isoperla sp.*	1	0.41	0.75	0.55	0.002	2	0.23	0.8	0.43	0.045
Insecta	Trichoptera	*Micrasema longulum*	1	0.44	0.41	0.43	0.017	1	0.77	0.23	0.42	0.047 "
Insecta	Plecoptera	*Protonemura sp.*	1	0.37	0.53	0.44	0.04	1	0.49	0.65	0.56	0.003
Insecta	Plecoptera	*Siphonoperla sp.*	1	0.34	0.75	0.5	0.005	1	0.7	0.45	0.56	0.002
Insecta	Ephemeroptera	*Ephemera danica*	2	0.26	0.77	0.45	0.027	5	0.41	1	0.64	0.002
Insecta	Plecoptera	*Leuctra sp.*	2	0.28	0.73	0.45	0.038	1	0.28	0.97	0.52	0.001
Insecta	Ephemeroptera	*Rhithrogena semicolorata-*Gr	2	0.28	0.91	0.5	0.001	4	0.32	0.56	0.43	0.034
Insecta	Ephemeroptera	*Epeorus assimilis*	2	0.38	0.73	0.53	0.003	1	0.35	0.52	0.43	0.024

Class	Order	Taxon	North Rhine-Westphalia					Thuringia				
			EC change gradient categories	A[a]	B[b]	IndVal	p value	EC change gradient categories	A[a]	B[b]	IndVal	p value
Gastro-poda	Sorbeoconcha	*Potamopyrgus antipodarum*	7	0.27	0.86	0.49	0.006	8	0.55	0.62	0.58	0.003

[a] proportion invertebrate are found in a single EC change gradient category.

[b] proportion an invertebrate occurs in all samples in that category.

DECLARATION

I, the author of this work, declare that this pHD thesis entitled ‘Pesticide and salinisation, two stressors of freshwater ecosystem’ has been composed by myself. The work has not be submitted at any university and other tertiary institution for any other degree or professional qualification. I confirm that the work submitted is my own, except where work which has formed part of jointlyauthored publications has been included. My contribution and those of the other authors to this work have been explicitly indicated clearly and acknowledged. Further, I have acknowledged all sources used and have cited these in the reference section.

I am aware that a violation of the above mentioned points can have legal consequences including the withdrawal of the doctoral degree.

Landau, 28.08.2020

Signature of student

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- **Le TDH**, Kattwinkel M, Schützenmeister K, Olson JR, Hawkins CP, Schäfer RB. 2019 Predicting current and future background ion concentrations in German surface water under climate change. Phil. Trans. R. Soc. B 374:20180004 http://dx.doi.org/10.1098/rstb.2018.0004
- **Le TDH**, Schreiner V, Kattwinkel M, Schäfer RB. 2020. Invertebrate turnover along gradients of anthropogenic salinisation in rivers of two German regions. *Science of the Total Environment. Volume 753, issue 141986, page 1-9* https://doi.org/10.1016/j.scitotenv.2020.141986
- Dinh, Q.T., Liang, D., Thu, T. thi A., **Le, T.D.H.**, Vuong, N.D.T., Pham, V.T., 2018.

  Spatial prediction of saline and sodic soils in rice–shrimp farming land by using integrated artificial neural network/regression model and kriging. Archives of Agronomy and Soil Science. https://doi.org/10.1080/03650340.2017.1352088

Research skills

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- Statistical modeling

Language

- Vietnamese
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- SETAC Brussels – SETAC Europe 27th Annual Meeting 2017 on ‘Environmental Quality Through Transdisciplinary Collaboration’

  Poster: Contribution of waste water treatment plants to pesticide toxicity in agriculture catchments
- SETAC GLB 2017 - German Language Branch on’ Society of Environmental Toxicology and Chemistry’

  Presentation: Contribution of waste water treatment plants to pesticide toxicity in agriculture catchments
- EGU Vienna - European Geoscience Union Gerneral Assembly 2018

Poster: Forecasting ion concentrations in surface waters under global environmental change

- SEFS-11 Symposium for European Freshwater Science, Croatia, 2019

  Presentation: Predicting current and future background conductivity in German surface water under climate change and ecological consequences