REACTIVE NITROGEN DYNAMICS IN STREAMS
CASE STUDY IN THE AGRICULTURAL UPPER ROXO CATCHMENT, PORTUGAL

Mariela Andrea Yevenes Burgos
Examining committee:

Prof.dr.ir.Z (Bob) Su University of Twente
Prof.dr.Victor Jetten University of Twente
Dr.A.A.Voinov University of Twente
Prof.dr.ir.A.van Griensven Free University of Brussels (VUB)
Priv.Doz.Dr.V.Geissen University of Bonn, Germany

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DISSERTATION

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Mariela Andrea Yevenes Burgos

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Prof. dr. ing. Wouter Verhoef, promoter
Dr. Chris Mannaerts assistant promoter
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Chapter 1

General Introduction
1.1 Background: Reactive Nitrogen

Nitrogen (N) is a vital nutrient for life that goes through many transformations in freshwater ecosystems at catchment scale. Nitrogen is the most abundant gas in the atmosphere, in order to be useful for most living organisms. However, it must be transformed into reactive forms, typically like nitrate, ammonium, ammonia, and organic nitrogen, amongst others. Historical and excessive use of artificial fertilizers and fossil fuels has intensely transformed the global nitrogen budget (Boyer et al., 2006). Most studies in the past made us aware of the dangers of releasing CO$_2$ into the environment, but it is known now that too much nitrogen release is harmful too. Some studies suggested that the rate at which humans add reactive nitrogen to the global budget has risen intensely; nitrogen increases are five times larger than observed atmospheric CO$_2$ increases over the last 50 years (Galloway et al., 2004; Davidson and Seitzinger, 2006; Gruber and Galloway, 2008). Anthropogenic activities are nowadays responsible for more than doubling of the global reactive N, which creates more than 157 Tg yr$^{-1}$, whereas all other natural processes only produce 125 Tg yr$^{-1}$ (Galloway et al., 2004).

The surplus of reactive N is a problem when it exceeds the 10 mg N L$^{-1}$, the maximum permissible upper limit concentration of nitrate in water established by the European directive 80/778/EEC (Europe directive, 2000). This excess of reactive N causes a cascade of effects that eventually cause degraded water quality (Smith, 2003; Galloway et al., 2003). Large-scale reactive N budgets show that about 20–25% of the nitrogen to the environment is transported by rivers and streams, which indicates that considerable sinks for reactive N must occur in the ecosystems (Boyer, 2006). Streams may be important sinks for reactive N for two reasons, i) the hydrological connections with terrestrial systems, and ii) the biological activity in sediments that support different biogeochemical processes where nitrogen can be transported or removed. Even intermittent and very small perennial streams may play an important role in regulating N inputs through biogeochemical processes, thereby mitigating the negative impacts (Soetaert and Meysman, 2012).

Nitrogen can enter streams from various point and nonpoint sources (e.g. runoff, groundwater, atmospheric deposition). Nitrate is the predominant form of reactive nitrogen in many streams, because it is highly soluble and readily
leached from soils. Ammonium is also important, but less prevalent in the water because it is incorporated into organic matter (OM), and adsorbed by predominantly negatively charged clay particles. Moreover, it is also rapidly nitrified in small streams (Craig et al., 2008). The amount of N delivered to downstream ecosystems is controlled by both permanent removal and temporary storage in the streams. Important losses of N take place mostly through denitrification processes. Denitrification is very important to the biosphere because it represents the primary pathway to reduce excess global reactive N (Davidson and Seitzinger, 2006). Denitrification is a microbial reduction process within the nitrogen cycle whereby nitrate (\(\text{NO}_3^-\)) and nitrite (\(\text{NO}_2^-\)) are reduced to \(\text{N}_2\) gas and returned to the atmosphere (Feast et al., 1998, Laverman et al., 2007). This process occurs predominantly in low \(\text{O}_2\) concentrations, in the presence of denitrifying bacteria and in suitable organic or inorganic electron donors.

In spite of denitrification as a natural efficient removal mechanism, the concentration of inorganic N has increased in many European streams (Feast et al., 1998; Kronvang et al., 2001a; Wade et al., 2002; Whitehead et al., 2006). Several studies have reviewed denitrification activity in streams and reservoirs (Saunders and Kalff, 2001a; Schaller et al., 2004; Royer et al., 2004; Piña-Ochoa and Álvarez-Cobelas, 2006). In stream waters, characterized by high concentrations of nitrate, denitrification may improve surface-water quality and afterwards compensate the negative effects of nitrogen enrichment (Ventullo and Rowe, 1982; Holmes et al., 1996). Nonetheless, in waters with low concentrations of nitrate and where nitrogen is limiting to algae and cyanobacteria, additional loss of nitrate through denitrification can negatively affect ecosystem production (Rysgaard et al., 1994). Therefore, understanding reactive nitrogen dynamics in streams is fundamental to control nitrogen levels and availability in drinking water sources (Otero, 2007). To address this issue, interdisciplinary approaches are needed to provide integral research efforts leading to greater understanding of aquatic biogeochemistry, and in employing tools as stable isotope geochemistry, eco-hydrological and geochemical modelling (Davidson and Seitzinger, 2006; Bernhardt and Palmer, 2007; Tetzlaff et al., 2007; Craig et al., 2008). Of all these methods used to study eco-hydrological and biogeochemical processes in catchment’s streams, tracers can provide best insights related to the origin and pathways of water movement and
biogeochemical processes. Both are among the few truly integrated measurements of watershed functioning.

1.2 Stable isotopes as a tool for tracing nitrogen sources

Isotopes are different forms of the same chemical element that have different number of neutrons in the nuclei, but all isotopes of an element have the same number of electrons and protons (Hoefs, 1997, Kendall and McDonnell, 1998). Stable isotopes do not decompose over time, and among them are several hydrogen, oxygen, nitrogen, carbon and sulfur isotopes. The use of stable isotopes to solve hydrological and biogeochemical problems in ecosystem analysis has increased rapidly. Because stable isotope data have contributed to both source-sink (tracer) and processes information (Peterson and Fry, 1987), and reflect the characteristic reactions taking place along specific flowpaths (Kendall and McDonnell, 1998), they are ideally suited to increase our understanding of water origin and element cycles in ecosystems.

Stable isotope ratios usually are expressed in delta notation ($\delta$) with respect to an isotope standard. Therefore, samples containing an N isotope ratio close to that of air will have $\delta^{15}$N values near zero (Mariotti, 1984a; Talbot, 2001). Numerous studies have been published over the last 20 years that deal with applications of environmental isotopes in hydrological investigations (Fritz and Fontes 1980; Clark and Fritz, 1997; Kendall and McDonnell, 1998; Rodgers et al., 2005; Wankel et al., 2006). Dual isotope studies have proven quite useful for source identification in some surface-water and groundwater studies (Böttcher et al., 1990; Wassenaar, 1995; Kendall et al., 1996; Kendall and McDonnell, 1998). A dual isotopic approach simultaneously with analysis of both N and O isotopes, offers an additional tool to investigate sources and N cycling. Furthermore, it may result in better understanding of the ecosystem (Kendall and McDonnell, 1998; Wankel et al., 2006).

1.3 Nitrogen dynamics and eco-hydrological models

Eco-hydrological models are integrative estimation tools useful in describing the eco-hydrological and biogeochemical state of a stream or river basin system. They help to predict the change in this state when certain initial conditions are altered and also for the assessment of the nitrogen dynamics due to non-point and point sources in streams or rivers (Tetzlaff et al., 2007; Krysanova and
Arnold, 2008). Particularly, in recent years, river water quality modelling has risen sharply because of the increasing governmental concern for river and stream water quality. Environmental guidelines such as the EU Water Framework Directive or the EU Nitrate Directive entail modelling practices to assess water quality to improve the ecology of streams and river basins (Lindenschmidt, 2006).

Streams or river networks have a hierarchical structure and natural boundaries, and can be considered as natural integrator in a catchment. This is an important reason why streams or river networks represent a suitable scale for integrated eco-hydrological modelling. Stream basin models usually include first a hydrological sub-model as a basic framework, and second a biogeochemical component (i.e. nutrients) considered for interactions among processes; e.g. water and nutrient drivers for plant growth and nutrient transport with water. Usually, climate and land-use related parameters are treated as external drivers, and vertical and lateral fluxes of water and nutrients in catchments are modelled separately (Krisanova and Arnold, 2008).

There are different classifications of river basin models and the differentiating principle could be the modelling approach or the scale of model application. Eco-hydrological river basin models are based on mathematical descriptions of physical, biogeochemical and hydrochemical processes by combining elements of both a physical and conceptual semi-empirical nature, and include a reasonable spatial disaggregation scheme e.g. sub-basins and for hydrological response units or HRUs (Krysanova and Arnold, 2008). Such deterministic models may also include stochastic elements. An example of this is the SWAT (Soil and Water Assessment Tool) model (Arnold et al., 1998) which is a process-based modelling tool for river basins. This model was developed to evaluate the effects of alternative management decisions on water resources and diffuse pollution in mesoscale and large river basins. The SWAT model is a useful tool for nutrient loading simulations in rivers and streams at catchment scale. Diffuse entries from the agriculture resulting from fertilizers as well as punctual entries from e.g wastewater treatment plants can also be considered in the model set-up. The SWAT model is a continuous-time, semi-distributed, process-based river basin model. In this research, the SWAT model was used to analyse nitrogen dynamics in a study catchment.
1.4 Nitrogen processes estimation using reactive transport models

The concentrations of reactive nitrogen are altered by chemical and biological transformations, and physical processes such as adsorption and transport. Their fate can be predicted using reactive transport models (RTM) that describe reaction and advective and dispersive movement of these components in their natural environment (Lichtner, 1985; Boudreau, 1997; Steefel et al., 2005; Runkel, 2007; Soetaert and Meysman, 2012). RTM have emerged as an essential diagnostic tool for the quantitative analysis of the biogeochemical functioning of aquatic environments. In particular, these models are crucial for the assessment of the fate of contaminants and the interpretation of the distribution of reactive chemical species in e.g. stream water or aquatic sediments (Soetaert et al., 1996; Soetaert and Meysman, 2012). Since RTM can be used as a diagnostic tool, reactive transport models also provide a platform for testing hypotheses derived from experimental observations or from the theoretical knowledge of biological, chemical or physical processes. Therefore, these models bridge the gap between fundamental, process-oriented research and applied research, along with the gap between results from laboratory experiments and observations in the field (e.g., contaminated sites). When combined with experimental or field data, these models can be used to extract rate parameters, give insight into the relative importance of processes that affect concentration distributions and quantify process rates that are often difficult or impossible to measure (Reichert, 1994; Soetaert et al., 1996; Chapra, 1997; Meysman et al., 2003; Runkel, 2007; Rasmussen et al., 2009).

1.5 Chlorophyll mapping using remote sensing

An increased nutrient concentration in freshwater often leads to a proliferation in chlorophyll (Chla) levels because of increased algal biomass in the water. Therefore, it is well known that Chla is a good indicator of the trophic state of aquatic ecosystems. Remote sensing-based methods can be used in estimating chlorophyll values (Lathrop and Liljesand 1986; Cox et al. 1998; Östlund et al. 2001; Kloiber et al. 2002; Koponen et al. 2002; Vignolo et al. 2006; Wang et al. 2006; Alparslan et al. 2007; Mancino et al. 2009). However, effective remote retrieval of Chla is a major challenge in eutrophic and turbid waters, due to the mixing of chlorophyll and suspended sediment that both affect the water
radiance spectrum and which may result in the wrong estimation of chlorophyll (Zhang et al., 2009).

The water radiance spectrum is a mixture because it is affected by multi-components of water quality (i.e. chlorophyll, suspended sediment, among others). Therefore, mixing spectral models are useful for trying to separate the different components in the water. Most of the studies have primarily involved this method in water quality monitoring in low turbid waters (Novo et al., 2006; Alcantara et al., 2009). A linear mixing approach was applied to derive accurate estimates of chlorophyll from Landsat Thematic Mapper imagery in Lake Balaton in Hungary (Tyler et al., 2006). Alcantara et al., (2009) evaluated the effectiveness of the spectral unmixing model for mapping the turbidity in an Amazon Floodplain Lake (Curuai Lake) using MODIS data. Shen and Verhoef, (2010) originally proposed a method to disentangle the interference of local atmospheric haze variations from sediment variations in the water. Spectral mixing models can be used to decompose the measured spectrum into components based on known endmember spectra of these components.

### 1.6 Problem Statement and Objectives

The upper Roxo catchment (352 km²) lies in an important agricultural region of the South of Portugal (Figure 1.1). Within this catchment is the Roxo dam (Albufeira do Roxo), which is the source of irrigation and domestic water supply to the Beja and Aljustrel towns. Water quality of this reservoir has a high economic importance in the catchment (Nunes et al., 2004). In this context, a significant part of the total nutrient load is discharged in the catchment through streams. Historical nitrogen loadings in the catchment and eutrophication of the Roxo reservoir have stimulated great attention to the importance of running waters in removing nitrogen waste. Therefore, there is concern over the potential impact of the nitrogen sources in the freshwater and the role of denitrification in streams.
Therefore, the main statement of this research is to analyse nitrogen sources and denitrification processes in the main streams and reservoir of the catchment using eco-hydrological modelling, spatial mixing patterns of different sources (groundwater, precipitation, surface inflow, etc.), along pathways. Analysis of bulk samples of nitrogen and oxygen isotopes and biogeochemical modelling and exploration of spectral mixing analysis tools were used to help in the biogeochemical assessment.

This research project has the following objectives:

1. To estimate the nitrate exports by streamflow of an agricultural catchment to a receiving drinking water reservoir, and to assess the relative importance of environmental factors such as climate, rainfall, streamflow, land-use and agricultural practices, including fertilizer use; and to infer hydrological and biogeochemical processes controlling the stream nitrate dynamics using an eco-hydrological modelling approach.

2. To assess whether a spatial sample-based analysis, using several seasonal observation campaigns, can be used as a data source for conducting end-member mixing analysis, using isotope and hydrochemical signatures to
decipher the water and dissolved chemical (nitrate) origins in the streamflow of a catchment and the inflow in a reservoir.

(3) To investigate the transport and fate of nitrate in the streams of an agricultural catchment through the combined use of the stable isotopes of nitrate and a 1-D reactive transport model; more specifically to simulate the reactive transport and behaviour of nitrate, ammonium and oxygen in the stream flow. Differences in nitrate concentrations and isotopic signatures from the upstream source to the receiving reservoir were compared, in order to detect potential hot spots of denitrification and/or cold spots of nitrification.

(4) To map, using remotes sensing imagery, chlorophyll-a and sediment levels in the eutrophic and turbid Roxo reservoir and to demonstrate the applicability of a chlorophyll-a (Chla) retrieval algorithm based on a linear spectral mixing model; and to link historical chlorophyll-a levels with the nutrient status of the reservoir using a biogeochemical nutrient limitation approach.

### 1.7 Structure of the Thesis

The different research parts of this thesis were grouped in six chapters. In **Chapter 1** the background, the problem statement and the objectives of this thesis are presented. The importance and biogeochemical relevance of nitrogen (N) dynamics in streams focusing on agricultural areas are introduces. The chapter further presents recent advances and challenges in the study of reactive nitrogen dynamics in streams tackled by different tools such as using stable isotopes, eco-hydrological and biogeochemical modelling and remote sensing, highlighting on reactive nitrogen dynamics, denitrification and nitrogen availability.

In **Chapter 2**, through the use of an eco-hydrological model (SWAT model), an eco-hydrological and biogeochemical baseline is established to predict the nitrate budget and export in the streams of the Roxo catchment. This chapter is based on an eight years period of historical data (climatic, chemical and physical). SWAT model calibration and validation are applied to analyse nitrate exports by streamflow from small streams to a main water reservoir (Roxo reservoir).
In **Chapter 3**, water and dissolved chemical (nitrate) origins in the streamflow are deciphered, using limited data from different seasons obtained in three field campaigns (2008-2009). Deuterium and oxygen stable isotope values and hydrochemical signatures from shallow groundwater, pore water, rainfall and stream water are used to conduct an end-member mixing analysis (EMMA) and exploratory analysis to assess nitrate and water origins.

**Chapter 4** analyses denitrification processes in the agricultural streams using nitrogen and oxygen stable isotopes, including 1D reactive transport model building, development and the calibration process using the R program. This study was conducted for the stream water of the streams and considered several biogeochemical, hydrological and physical factors that are influencing the streams’ condition.

In **Chapter 5**, a spectral mixing model and Landsat TM imagery are used to map chlorophyll and sediment levels in the Roxo reservoir and to compare them with historical nutrient trends to assess the nutrient availability/limitation in the reservoir. This chapter introduces a linear unmixing method to obtain the spatial chlorophyll-a distribution and nutrient availability in the reservoir.

Finally in **Chapter 6** conclusions, limitations and future work are presented. This chapter involves the synthesis of the different issues analysed in this thesis, including nitrate export using eco-hydrological modelling, stable isotopes composition and geochemical modelling using two biogeochemical pathways, denitrification and nitrification and remote sensing techniques to map chlorophyll and sediment in an eutrophic reservoir (Roxo reservoir). It further embraces conclusions and future work.
Chapter 2

Seasonal and land use impacts on the nitrate budget and export of a mesoscale catchment in Southern Portugal
Abstract

Stream nitrate nitrogen exports are an important indicator of agricultural impacts on aquatic health in catchments. An eco-hydrological catchment modelling approach, using the SWAT model driven by detailed field data, was used to analyse the nitrate export and the components of the nitrogen budget of the 352 km² upper Roxo river catchment in Southern Portugal. A detailed eight-year record (2001–2008) of the monitoring of weather, reservoir inflow, stream biogeochemistry, soils, in-stream and groundwater quality, and fertilizer application was used to calibrate and validate the model. Results indicated that monthly nitrate loadings varied from 0.02 to 2.48 kg N ha⁻¹ during summer and between 0.03 and 14 kg N ha⁻¹ during late autumn and winter. Stream nitrate values, ranging from 1.5 to 16.5 mg N L⁻¹, were strongly related to extreme rainfall occurrences and wet periods. Nitrate budget components at the sub-catchment level enabled evaluation of the impacts of the various processes affecting the nitrate nitrogen pool of the catchment. Besides high fertilizer inputs for annual crops, it was shown that biological nitrogen fixation and wet deposition by rainfall should be accounted for in input balances. Where denitrification naturally reduces nitrate levels in soils, streams and the reservoir, the largest contribution to stream nitrate originates from leached soil nitrate reappearing in groundwater baseflow, compared with less than 2% from direct surface runoff during high rainfall events. A fertilizer reduction scenario was effectively implemented to evaluate remedial nitrate control policies in accordance with the European Nitrate and Water Framework Directives. Agricultural practices and seasonal weather fluctuations were the main reasons for temporal variations in nitrate export via small streams to the main reservoir.

2.1 Introduction

Nitrogen levels in streamflow are important indicators of environmental catchment conditions (Arheimer and Brandt, 1998; Mulholland et al., 2005; Piatek et al., 2009). A multitude of human activities, such as agricultural practices or urban residual waste water effluent releases, may produce an excess of nitrogen supply in a catchment, and can lead to increased nitrogen losses, especially in the form of nitrate nitrogen (nitrate), thus disturbing and impacting the water quality of ecosystems (Ventura et al., 2008). Concern about nitrate impacts on freshwater bodies from activities such as agriculture dates back more than 40 years ago, when the Commission of the European Community (CEC) became interested in maximizing the fertilizer potential of animal slurry applied to agricultural areas (Sluijsmans, 1978). Nowadays, it is still a concern in the European Water Framework Directive, whereby several agriculture-dominated regions across Europe have been classified in the European Nitrate Directive 91/676/CEE as areas vulnerable to nitrate contamination from agricultural sources.

The Roxo river is an upper tributary of the Sado basin and is located in the important agricultural Alentejo region of Southern Portugal. The catchment area is within the zone of influence of the large Alqueva dam and reservoir, and has been classified as a vulnerable zone since 2006 according to the European Nitrate Directive 91/676/CEE. The Roxo upper catchment (352 km²) drains into a reservoir, which is the main source of the domestic water supply for Beja city, as well as the water supply for the local mining industry and some important irrigation areas (ABROXO, 2009). The reservoir has, however, been under considerable water stress for several years owing to the combination of interannual weather variability that affects natural rainfall supply, increased water consumption, and contamination threats of varying origin (UNEP, 1997). There is serious concern among the local and regional authorities regarding the Roxo reservoir, related to both water quantity and quality.

Several field studies and data from the Roxo catchment have indicated high nitrate concentrations, around 15 mg N L⁻¹ in small streams and shallow groundwater (Chisha, 2003 Gurung, 2005; Vithanage, 2009). Gurung (2005) suggested that the Roxo reservoir is a hypertrophic system, because maximum nitrate concentrations of 14 mg N L⁻¹ and high values for other eutrophication
Seasonal and land use impacts on nitrate budget

indicators such as phosphorus and chlorophyll-a were regularly observed in the reservoir. Vithanage (2009) recorded NO$_3^-$ levels ranging from 2 to 13 mg N L$^{-1}$ in streams located in the southern part of the catchment, which in fact significantly exceeds the nitrate levels (5.65 mg N L$^{-1}$) established by the European Water Framework Directive (2000/60/EC). However, it is known that only a small percentage of the net nitrogen pool in a catchment is generally exported to streams (Boyer et al., 2002), while the rest is retained or lost in the watershed system through denitrification or volatilization into the atmosphere before reaching the water body (Filoso et al., 2003). Nevertheless, nitrate export studies remain important because excess nitrogen inputs in a water body can dramatically increase primary productivity and decrease the water quality of the impoundment (Caraco and Cole, 2001; Alvarez-Cobelas et al., 2008). Observation of high nitrate concentrations in natural waters may also be indicative of the possible presence and flows of other nutrients (e.g. phosphorus) or contaminants (e.g. pesticides). Several studies consider an observation period of five years or more as sufficient for nitrate export studies, since this enables the spatial and temporal variability involved in the seasonal periodicity of nitrogen fluxes to be captured (Alvarez-Cobelas et al., 2008). Local medium-term studies have proved to be better than single-year analyses when it comes to understanding the controlling factors of catchment nitrogen fluxes (Schilling and Zhang, 2004; Alvarez-Cobelas et al., 2008). The nitrate export of a catchment is affected by environmental factors such as ambient temperature, rainfall, runoff, streamflow, soils and land use, including agricultural practices such as fertilizer application and potential point sources (Schilling and Zhang, 2004). Catchment studies carried out in Europe have reported nitrate export values ranging from 0.4 kg N ha$^{-1}$ to 17 kg N ha$^{-1}$ yr$^{-1}$ (Isidoro et al., 2006; Alvarez-Cobelas et al., 2008). With regard to the Roxo catchment in particular, mineral fertilizer, manure and residual waste water disposal are potentially major non-point sources of excess nitrogen.

The aim of this study is to estimate the nitrate exports by streamflow from the small streams to a water reservoir in the Roxo catchment in Southern Portugal, in order to assess the relative importance of environmental factors such as rainfall distribution, streamflow, land use and agricultural practices affecting nitrate loadings and losses in a mesoscale catchment, and ultimately to predict the hydrological or biogeochemical processes controlling the stream nitrate...
dynamics. The Soil and Water Assessment Tool or SWAT 2005 eco-
hydrological model (Neitsch et al., 2005) was used for this purpose, using an
eight-year period (2001–2008) of monitoring data. The SWAT model has been
extensively used to determine rainfall-runoff responses and nutrient loadings in
streamflow and biogeochemical processes in moderately and poorly gauged
catchments (Pohlert et al., 2005; Hu et al., 2007; Lam et al., 2009).

2.2 Materials and Methods

2.2.1 Study Area

The study area is located in the Roxo catchment in the Beja district of Alentejo
province, Southern Portugal (37º46’44’’N to N latitude and 7º5’47’’E to
8º12’24’’E longitude; Figure 2.1). With a catchment area of 352 km², it is
considered a mesoscale catchment. The topography varies from nearly flat to
gently sloping terrain, with elevations ranging from 123 m at the catchment
reservoir outlet to 280 m.a.s.l. near Beja city. Alentejo province alone yields
75% of Portugal’s total wheat production (Paralta and Oliveira, 2005). The
region and the Roxo catchment are dominated by agricultural activities. The
major crops produced in the region are winter wheat, maize, alfalfa and
sunflower as rotation crops, and olives, vineyards (grapes) and cork oak as
perennial agricultural crops (Table 2.1). Agricultural land covers more than
80% of the catchment. Winter wheat and alfalfa, as intensive crops, commonly
require around 100 kg N ha⁻¹ yr⁻¹ of fertilizer, whereas recommended nitrogen
fertilization for maize is around 150–200 kg N ha⁻¹ yr⁻¹ (Paralta and Oliveira,
2005; personal communication M. Varela of Centro Operativo e de Tecnologia
de Regadio (COTR) and R. Nobre of Escola Agraria do Beja, Portugal). For
olive and oak plantations, fertilizer application and amounts are quite variable,
and depend mainly on foliar analysis and tree age. However, an average
application of 100 kg N ha⁻¹ yr⁻¹ is common practice for olive orchards in the
production season (personal communication M. Varela of COTR). Fertilization
of range and grassland is negligible and is an uncommon practice in Portugal.
Some areas of natural forest and silvicultural activities are present in the south
of the catchment. Literature related to fertilizer use in eucalyptus plantations
indicates minimal use: about 60 kg N ha⁻¹ applied at the start of the plantations
(Filoso et al., 2003). Two other natural nitrogen input sources in the catchment
are biological nitrogen fixation by crops such as alfalfa, and atmospheric wet
deposition by rainfall. Water in the catchment drains into an artificial
impoundment, the Roxo reservoir (maximum volume approximately 108 m³), which was built in the early sixties and is used for municipal water supply to Beja city and its approximately 161,000 inhabitants, for the local mining industry, and for irrigation water supply to several areas (ABROXO, 2009). The irrigation water volume accumulated in Roxo reservoir is not used within the catchment, but is used to irrigate areas downstream of the reservoir. Water for crop irrigation in the catchment area comes from shallow groundwater, which is pumped to center pivot systems to irrigate crops such as alfalfa and maize (Table 2.1). The sewage waters from Beja city are channeled to a waste water treatment plant, before the residual waters are released into the Chamine-Pisoses streams in the upper part of the catchment. This also yields an additional and relatively constant nitrogen input and loading in the upper catchment stream network. The reservoir lake and riparian area cover an average area of 11.9 and 20 km², respectively, and represent 3.38 4% and 10.2% of the total catchment area. The long-term mean annual rainfall in the catchment region ranges from 500 to 550 mm. Soil survey using the FAO-UNESCO classification system identified four main soil types in the catchment: Luvisols, Litosols, Planosols and Vertisols (Sen and Gieske, 2005). The Luvisols cover 64% of the study area and are consequently the dominant soil type (Gamises, 2009). This soil type, with loam to clay loam texture, extends from the northeastern part to the southern part. Soil physical properties include texture, bulk density, available water capacity, saturated conductivity and organic carbon percentage (Table 2.2).
Table 2.1. Land use-land cover and crop management information in upper Roxo catchment

<table>
<thead>
<tr>
<th>Land use – Land cover</th>
<th>Crop information</th>
<th>Total area (km²)</th>
<th>Total area %</th>
<th>Fertilizer use</th>
<th>Fertilizer Type or NPK</th>
<th>Amount kg/ha</th>
<th>Timing / dates</th>
<th>Irrigation system</th>
<th>Irrigation volumes mm/period</th>
<th>Irrigation timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agricultural - Arable land</td>
<td>Maize (irrigated)</td>
<td>27.11</td>
<td>7.7</td>
<td>Planting</td>
<td>Boost</td>
<td>15-35-00</td>
<td>100</td>
<td>June-August</td>
<td>Center Pivot</td>
<td>250-550</td>
</tr>
<tr>
<td>Winter annual in rotation</td>
<td>Alfalfa (irrigated)</td>
<td>18.93</td>
<td>5.4</td>
<td>Planting</td>
<td>Nitro 32N</td>
<td>6-20-15</td>
<td>300</td>
<td>June-August</td>
<td>Center Pivot</td>
<td>230-300</td>
</tr>
<tr>
<td></td>
<td>Winter wheat or barley, bare fallow</td>
<td>68.72</td>
<td>19.5</td>
<td>Development</td>
<td>Nitro 7%</td>
<td>20-20-00</td>
<td>200</td>
<td>June-August</td>
<td>Center Pivot</td>
<td>230-300</td>
</tr>
<tr>
<td>Agricultural - Mixed crops</td>
<td>Summer annuals pasture, long fallow</td>
<td>129.41</td>
<td>36.8</td>
<td>SWAT auto-fertilization option</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agricultural - Permanent crops</td>
<td>Olive groves</td>
<td>25.33</td>
<td>7.2</td>
<td>SWAT auto-fertilization option</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vineyards</td>
<td>6.28</td>
<td>1.8</td>
<td>“</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cork oak</td>
<td>23.28</td>
<td>6.6</td>
<td>”</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water bodies</td>
<td>Ponds, reservoir</td>
<td>10.04</td>
<td>3.0</td>
<td>Not applicable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Semi-natural vegetation</td>
<td>Rangeland</td>
<td>14.41</td>
<td>4.1</td>
<td>Not applicable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forest land</td>
<td>Eucalyptus, Pinus spp</td>
<td>28.17</td>
<td>8.0</td>
<td>auto-fertilization option</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban fabric</td>
<td>Urban Low density</td>
<td>0.34</td>
<td>0.10</td>
<td>Not applicable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urban High density</td>
<td>0.52</td>
<td>0.15</td>
<td>Not applicable</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) Crop and irrigated areas based on ASTER satellite image land cover classification (July 2004) and field survey. Irrigation volumes variable as a function of crop, soil type and period (spring, summer); from observed pivot data (Aman, 2004).
We used measured soil properties at our own institution (Gökmen, 2006; Gamises, 2009) in combination with official Portuguese soil data and information (Cardoso, 1965).

### 2.2.2 Data Collection and nitrate export prediction

For this study, water quality data and information on nitrogen were collected from various sources. Groundwater nitrate (Paralta and Oliveira, 2005) and surface water hydrochemical and nutrient data were measured during several field campaigns between 2003 and 2009 (Chisha, 2003; Gurung, 2005; Mekonnen, 2005; Gökmen, 2006; Vithanage, 2009) and by the authors during the period 2008 and 2009. A comprehensive water quality monitoring dataset of the Roxo reservoir from the local water authorities (EMAS, 2008; SNIRH, 2008) was used for generating nitrate time series of the receiving Roxo reservoir water body.

![Figure 2.1. Location of the sampling points in the study area](image)

Daily records of precipitation, air temperature, solar radiation, wind and relative humidity were obtained from automatic weather stations located near the Aljustrel and Beja areas, and were used to generate the SWAT weather inputs (data source ref. COTR). Long-term climate data were used to create the weather generator parameter files (Neitsch et al., 2005). Missing data for the
model are automatically generated based on historical records (Hu et al., 2007). Daily catchment streamflow, assumed as being equivalent to the total reservoir inflow, was generated using an inverted reservoir water balance approach. An extensive detailed daily dataset from 2001 to 2008 of precipitation, evaporation, reservoir storage volume, historical daily reservoir water levels and water abstraction data (water for irrigation, drinking and industrial purposes) was made available by the Portuguese authorities (ABROXO, 2009). A reservoir mass balance method was used to estimate the total catchment streamflow volumes in the reservoir. This technique consisted of estimating the reservoir inflow from the variation over time in the storage volume of the reservoir and the total of all outflows and losses from the reservoir (Vithanage, 2009). These inflow volumes were used to derive the streamflow into the reservoir.

Information on agricultural practices such as general land management, crop rotation, fertilizer use, type and times of fertilizer application, planting and irrigation were obtained from COTR and Escola Agraria Superior de Beja (Table 2.1). In soils, nitrate is generally very reactive and can be added in different ways, either by rainfall, fertilizer or biological N fixation. Soil nitrate can be removed through various hydrological and biogeochemical processes, such as runoff, leaching, volatilization, denitrification or plant uptake (Neitsch et al., 2002). We estimated soil denitrification rates from laboratory experiments on the 12 soil units and land uses in the Roxo catchment.
Table 2.1. Soil properties of upper Roxo catchment

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Sample depth cm</th>
<th>Clay %</th>
<th>Silt %</th>
<th>Sand %</th>
<th>BD g cm$^{-3}$</th>
<th>AWC % (vol)</th>
<th>$K_{sat}$ mm h$^{-1}$</th>
<th>OC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cph</td>
<td>0-20</td>
<td>41.5</td>
<td>24.1</td>
<td>36.4</td>
<td>1.51</td>
<td>0.12</td>
<td>2.16</td>
<td>1.5</td>
</tr>
<tr>
<td>Bpc</td>
<td>0-20</td>
<td>41.9</td>
<td>23.6</td>
<td>29.8</td>
<td>1.87</td>
<td>0.12</td>
<td>0.78</td>
<td>0.9</td>
</tr>
<tr>
<td>Px</td>
<td>0-20</td>
<td>39.8</td>
<td>29.1</td>
<td>29.3</td>
<td>1.81</td>
<td>0.13</td>
<td>3.12</td>
<td>1.3</td>
</tr>
<tr>
<td>Vx</td>
<td>0-20</td>
<td>33.5</td>
<td>33.9</td>
<td>32.7</td>
<td>1.87</td>
<td>0.13</td>
<td>7.44</td>
<td>1.4</td>
</tr>
<tr>
<td>Sr</td>
<td>0-20</td>
<td>31.4</td>
<td>30.5</td>
<td>34.0</td>
<td>1.97</td>
<td>0.13</td>
<td>17.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Vc</td>
<td>0-20</td>
<td>35.5</td>
<td>22.6</td>
<td>40.9</td>
<td>1.54</td>
<td>0.12</td>
<td>7.56</td>
<td>1.2</td>
</tr>
<tr>
<td>Ah</td>
<td>0-20</td>
<td>44.8</td>
<td>21.6</td>
<td>33.7</td>
<td>1.52</td>
<td>0.12</td>
<td>1.02</td>
<td>0.9</td>
</tr>
<tr>
<td>Ps</td>
<td>0-20</td>
<td>27.0</td>
<td>36.0</td>
<td>37</td>
<td>1.66</td>
<td>0.13</td>
<td>5.52</td>
<td>0.6</td>
</tr>
<tr>
<td>Sp</td>
<td>0-20</td>
<td>36.6</td>
<td>41.0</td>
<td>22.5</td>
<td>1.79</td>
<td>0.15</td>
<td>7.68</td>
<td>0.7</td>
</tr>
<tr>
<td>Pxd</td>
<td>0-20</td>
<td>21.8</td>
<td>32.4</td>
<td>43.6</td>
<td>1.59</td>
<td>0.13</td>
<td>11.8</td>
<td>0.7</td>
</tr>
<tr>
<td>Pb</td>
<td>0-20</td>
<td>37.4</td>
<td>31.4</td>
<td>30.2</td>
<td>1.55</td>
<td>0.12</td>
<td>4.08</td>
<td>0.8</td>
</tr>
<tr>
<td>Pag</td>
<td>0-20</td>
<td>25.3</td>
<td>34.8</td>
<td>39.9</td>
<td>1.69</td>
<td>0.12</td>
<td>5.88</td>
<td>0.8</td>
</tr>
</tbody>
</table>

BD: bulk density; AWC: available water capacity; K: Hydraulic conductivity; OC: organic carbon. * Soil unit code from Cardoso (1965): Cph, Vertisol – calcareous black; strongly decarbonated Bpc, Vertisols – calcareous black, strongly decarbonated; Px, brown Mediterranean soils from non-calcereous rocks; Vx, red-yellow Mediterranean soils from non-calcereous; Sr, red-yellow Mediterranean soil from non-calcereous normal; Vc, red calcareous soils – red calcareous soils of semi-arid climate; Ah, humic Vertisol; Ps, unsaturated hydromorphic soils – with eluvial horizon – Planosols; Sp, hydromorphic soils – hydromorphic organic soils; Pxd, brown Mediterranean soils from non-calcereous rocks – normals; Pb, hydromorphic soils – without alluvial horizon – not strongly unsaturated; Pag, hydromorphic-brown Mediterranean soils.

We followed an indirect approach of anaerobic incubation of soils adjusted with potassium nitrate ($\text{KNO}_3$) without the addition of organic carbon (Yeomans et al., 1992). Groundwater nitrate concentration and physical parameters were also measured during winter and summer 2008–2009, and obtained from literature for summer 2003 and 2005 (Paralta and Oliveira, 2005).

Streamflow, the catchment nitrogen budget and nitrate fluxes in runoff were estimated for eight years (2001–2008) using the SWAT 2005 model (Neitsch et al., 2005). The SWAT model was developed by the US, Department of Agriculture and the University of Texas (Arnold et al., 1998), and is a spatially distributed, physically based hydrological model that can operate on a daily, monthly, or annual time step. The data preprocessing is achieved in a two-step approach. First, the sub-catchments, streams, channel length and hill slopes are derived from a digital elevation model. Second, land use and soil classes are overlaid and multiple hydrological response units are generated within each
sub-catchment. The climatic variables required by SWAT consist of daily precipitation, minimum and maximum air temperature, solar radiation, wind speed, and relative humidity. The model allows the input of observed daily records from weather stations or the generation of weather and climate variables using a built-in weather simulator. This generator uses long-term monthly means of the weather variables. The SWAT model includes large U.S. climate, soil and land cover–land use databases. To run the model in other regions of the world, it is necessary to create additional database records for weather, soils and land uses, using regional and local data. In this analysis, we generated all the parameters required to run the program as described by Shanti et al. (2001), Chu et al. (2004), Hu et al. (2007) and Bosch (2008). The catchment was divided into 13 sub-catchments based on a threshold flow accumulation area of 1000 ha. The combination of 10 different land uses, 12 soil units, and slope steepness resulted in the 243 hydrological response units used in the analysis.

Flow data from the period 2001–2004 were used for calibration, whereas data from 2005 to 2008 were used for validation using a monthly time step. Nitrate calibration and validation were carried out using datasets from 2003 to 2005 and from 2005 to 2008, respectively. The streamflow calibration process was completed by varying several SWAT hydrological parameters within their acceptable ranges (Table 2.3) in order to adjust the model predicted monthly baseflow, streamflow and nitrate data. The SCS curve number (CN) method was selected to generate runoff volumes from rainfall. The CN values were initially parameterized using a combination of land use and soil properties. We used the standard procedures (USDA Natural Resources Conservation Service, 1986) to determine soil hydrological group and CN values. The percolation component used a storage routing technique to predict flow through each soil layer in the root zone. Lateral subsurface flow in the soil profile is calculated simultaneously with percolation. Groundwater flow contribution to total streamflow is simulated by routing a shallow aquifer storage component to the stream (Arnold et al., 2000).
Table 2.2. SWAT model parameter calibration and sensitivity (Upper Roxo catchment)

<table>
<thead>
<tr>
<th>Parameter description</th>
<th>SWAT Code</th>
<th>Parameter sensitivity</th>
<th>Initial value</th>
<th>Adjusted value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCS Runoff Curve Number</td>
<td>CN2</td>
<td>1.86</td>
<td>Variable by HRU</td>
<td>[63-84] value range</td>
</tr>
<tr>
<td>Threshold depth of water in shallow aquifer [mm]</td>
<td>GWQMNN</td>
<td>0.77</td>
<td>0.1</td>
<td>3.0</td>
</tr>
<tr>
<td>Threshold capillary rise shallow aquifer [mm]</td>
<td>REVAPMM</td>
<td>0.66</td>
<td>0.1</td>
<td>0.5</td>
</tr>
<tr>
<td>Baseflow recession alpha factor (day)</td>
<td>Alpha_BF</td>
<td>0.21</td>
<td>0.05</td>
<td>0.11</td>
</tr>
<tr>
<td>Soil evaporation compensation factor [-]</td>
<td>ESCO</td>
<td>0.21</td>
<td>1.0</td>
<td>0.70</td>
</tr>
<tr>
<td>Soil available water capacity (in mm H2O)</td>
<td>SOL_AWC</td>
<td>0.10</td>
<td>Variable by soil unit</td>
<td>[0.11-0.16] value range</td>
</tr>
<tr>
<td>Soil depth of layers (m)</td>
<td>SOL_Z</td>
<td>0.08</td>
<td>Variable by soil unit</td>
<td>[0.4-1.2] value range</td>
</tr>
<tr>
<td>Soil saturated hydraulic conductivity (mm/hr)</td>
<td>SOL_K</td>
<td>0.07</td>
<td>Variable by soil unit</td>
<td>[1.05-11.7] value range</td>
</tr>
<tr>
<td>Leaf area index for crop</td>
<td>BLAI</td>
<td>0.03</td>
<td>Variable by HRU</td>
<td>[0.0-5.4] value range</td>
</tr>
<tr>
<td>Surface runoff lag coefficient (day)</td>
<td>SURLAG</td>
<td>0.02</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Deep aquifer percolation fraction (-)</td>
<td>RCHRG_DP</td>
<td>0.02</td>
<td>0.05</td>
<td>0.10</td>
</tr>
<tr>
<td>Delay time (day)</td>
<td>GW_DELAY</td>
<td>0.01</td>
<td>20</td>
<td>12</td>
</tr>
<tr>
<td>Plant water uptake compensation factor [-]</td>
<td>EPCO</td>
<td>0.01</td>
<td>1.0</td>
<td>0.75</td>
</tr>
<tr>
<td>Shallow aquifer initial storage [mm]</td>
<td>SHALL_ST</td>
<td>0.01</td>
<td>0.1</td>
<td>200.0</td>
</tr>
<tr>
<td>Deep aquifer initial storage [mm]</td>
<td>DA_ST</td>
<td>0.01</td>
<td>1000.0</td>
<td>1000.0</td>
</tr>
<tr>
<td>Fertilizer application fraction in topsoil 10 mm</td>
<td>FERT_LY</td>
<td>n.a.</td>
<td>0.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Rainfall nitrate (mg N L⁻¹)</td>
<td>CNR</td>
<td>n.a.</td>
<td>0.5</td>
<td>0.48</td>
</tr>
<tr>
<td>Denitrification rate coefficient (fraction)</td>
<td>CDN</td>
<td>n.a.</td>
<td>0.1</td>
<td>0.20</td>
</tr>
</tbody>
</table>

a : Mean parameter sensitivity as obtained from SWAT model sensitivity analysis using Latin Hypercube method (van Griensven et al, 2006)

We verified baseflow using other refereed data and several field observations and measurements (reservoir inflows) made during the 2001–2008 simulation period by various authors (Paralta, 2001; Mekonnen, 2005). We added one
complete parameter dataset for olive orchards to the crop inputs. This crop type was not included in the standard SWAT 2005 land use database.

The insertion of this land use was performed by R. Srinivasan’s SWAT development group at the Spatial Sciences Laboratory of Texas A&M University, College Station TX, USA (personal communication). Automatic calibration was selected mainly because manual calibration of the SWAT model for mesoscale catchments is not only tedious and time consuming but could also potentially lose final global outputs (Hu et al., 2007).

After each simulation, SWAT outputs were evaluated for goodness-of-fit using three model performance indicators: the Nash–Sutcliffe coefficient (\(\xi_{NS}\)), the coefficient of determination (R^2), and the deviation of data being evaluated, expressed as a percentage bias or PBIAS (Moriasi et al., 2007).

With regard to the nitrogen export data analysis, monthly nitrate loads were calculated based on total monthly streamflows multiplied by monthly nitrate concentration. Nitrate exports per unit area for each sub-catchment were estimated by evaluating the inputs versus outputs using the nitrate budget.

Three agricultural management scenarios were included. A first scenario evaluated the water and nitrogen budget, based on standard practices but without irrigation in the catchment. In a second scenario, we evaluated the effect of the within-basin pivot irrigation practices on the water and nitrate nitrogen budget of the catchment. A third fertilizer reduction scenario was also implemented. In this scenario, the original values for nitrogen fertilizer application were reduced by 20% for maize, winter wheat and alfalfa, to explore the impact of fertilizer level on the nitrate budget and the water quality in the catchment and reservoir. Nitrogen data for precipitation were derived from EUSAAR (European Supersites for Atmospheric Aerosol Research).

### 2.3 Results

Daily and monthly streamflow of the Roxo catchment was successfully simulated, calibrated and validated by the SWAT 2005 model (Figure 2.2). Monthly streamflow simulations were acceptable according to the statistical model performance measurements. Model prediction for calibration presented a Nash–Sutcliffe coefficient (\(\xi_{NS}\)) and the coefficient of determination (R^2) of
Seasonal and land use impacts on nitrate budget

0.65 and 0.60, respectively (Table 2.4). The simulation showed that the model was acceptable with respect to streamflow at the end of the summer (September–October).

Mean monthly streamflow during the full analysis period (calibration and validation) averaged 0.83 ±1.56 m³ s⁻¹, with the lowest monthly streamflow of 0.069 m³ s⁻¹ occurring in July 2005, and the highest monthly streamflow of 9.54 m³ s⁻¹ observed in November 2006 for the validation period. During autumn and winter months (e.g. November), high flows regularly occur after larger precipitation events (Figure 2.2).

Table 2.3. Values of test statistics for SWAT model calibration and validation for Roxo Catchment: PBIAS, ENS and regression coefficient of determination (R²) for monthly stream flow and nitrate load.

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulated mean flow (m³ s⁻¹)</td>
<td>0.88 ± 0.71</td>
<td>0.62 ± 1.23</td>
<td></td>
</tr>
<tr>
<td>PBIAS (% bias)</td>
<td>48</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>ENS (ξ Nash-Sutcliffe)</td>
<td>0.65</td>
<td>0.60</td>
<td></td>
</tr>
<tr>
<td>R² (coeff. of determination)</td>
<td>0.60</td>
<td>0.77</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulated nitrate load</td>
<td>6.24 ± 7.15</td>
<td>5.55 ± 8.16</td>
<td></td>
</tr>
<tr>
<td>PBIAS (% bias)</td>
<td>48</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>ENS (ξ Nash-Sutcliffe)</td>
<td>0.60</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>R² (coeff. of determination)</td>
<td>0.70</td>
<td>0.76</td>
<td></td>
</tr>
</tbody>
</table>

In May, after the wet season, crop irrigation starts and stream discharge adopts a baseflow recession regime of around 1.09±0.73 m³ s⁻¹ on average. Total average annual catchment precipitation over the study period was 518 ±48.9 mm. Significant rainfall periods were recorded during 2001, 2003 and 2006, with the highest values in 2006 (Figure 2.2). Annual precipitation for 2006 was 718 mm, with extreme rainfalls totalling 237 mm in November. Precipitation in spring and summer was close to zero. After calibration, the model predicted that, from the mean annual rainfall over the catchment area of 517.6 mm, 237.7 mm were removed through evapotranspiration, 48.8 mm were converted to direct surface runoff, and 237.4 mm percolated to the groundwater aquifer. From this initial drainage to the shallow aquifer, 9.2 mm re-entered the soil through capillary rise, 11.9 mm recharged the deep aquifer, and 216.3 mm appeared as baseflow in the stream network. Simulated mean annual catchment
water yield and Roxo reservoir inflow for the whole simulation period amounted to 265.1 mm (Table 2.5). Using calibrated model parameter data, the SWAT model successfully predicted nitrate load in the Roxo catchment (Figure 2.3). For the calibration, monthly nitrate values showed $\xi_{NS}$ and $R^2$ values of 0.60 and 0.70 (Table 2.4).

Figure 2.2. Observed versus simulated monthly streamflow in the Roxo catchment and monthly precipitation for the eight-year record spanning the calibration period (2001–2004) and the validation period (2005–2008).
Figure 2.3. Observed and simulated monthly nitrate loadings for the calibration period (2003–2005) and the validation period (2005–2008) and precipitation for the whole period.
Table 2.4. Annual water balance and nitrate budget components for agricultural management scenarios of the upper Roxo catchment.

### Annual basin water balance [in mm H₂O]

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Precipitation [mm]</th>
<th>Surface runoff [mm]</th>
<th>Total aquifer recharge [mm]</th>
<th>Shallow aquifer capillary rise [mm]</th>
<th>Deep aquifer Recharge [mm]</th>
<th>Basin water yield [mm]</th>
<th>Evapo-transpiration or ETa [mm]</th>
<th>Potential or PET [mm]</th>
<th>Irrigation volume [mm]</th>
<th>soil water balance [mm]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scenario a: No irrigation from aquifer</strong></td>
<td>Mean 4</td>
<td>517.6</td>
<td>48.8</td>
<td>237.4</td>
<td>9.2</td>
<td>11.9</td>
<td>216.3</td>
<td>265.1</td>
<td>237.7</td>
<td>1327.5</td>
</tr>
<tr>
<td><strong>Scenario b: Center pivot irrigation from aquifer on summer crops (example case: maize and alfalfa on 3.8% of area – see also Table 2.1)</strong></td>
<td>Mean 4</td>
<td>517.6</td>
<td>49.6</td>
<td>234.4</td>
<td>9.4</td>
<td>11.9</td>
<td>213.1</td>
<td>262.7</td>
<td>245.5</td>
<td>1327.5</td>
</tr>
</tbody>
</table>

### Annual basin area-averaged nitrate budget [in kg NO₃-N ha⁻¹ yr⁻¹]

<table>
<thead>
<tr>
<th>Scenario</th>
<th>NO₃ input by fertilizer</th>
<th>NO₃ input rainfall</th>
<th>NO₃ in by biological fixation</th>
<th>NO₃ to shallow aquifer</th>
<th>NO₃ to deep aquifer</th>
<th>NO₃ in aquifer baseflow</th>
<th>NO₃ in Surface runoff</th>
<th>NO₃ loss denitrification</th>
<th>NO₃ uptake by plants</th>
<th>NO₃ export by crop harvest</th>
<th>Soil NO₃ Balance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scenario a: No irrigation from aquifer</strong></td>
<td>Mean 4</td>
<td>75.4</td>
<td>2.6</td>
<td>1.4</td>
<td>54.9</td>
<td>7.4</td>
<td>47.5</td>
<td>0.3</td>
<td>7.1</td>
<td>43.3</td>
<td>8.9</td>
</tr>
<tr>
<td><strong>Scenario b: Center pivot irrigation from aquifer on summer crops (example case: maize and alfalfa on 3.8% of area – see also Table 2.1)</strong></td>
<td>Mean 4</td>
<td>80.2</td>
<td>2.6</td>
<td>1.4</td>
<td>55.4</td>
<td>7.5</td>
<td>47.7</td>
<td>0.3</td>
<td>7.2</td>
<td>45.6</td>
<td>9.1</td>
</tr>
<tr>
<td><strong>Scenario c: 20% Fertilizer reduction scenario (other conditions as standard practice scenario b)</strong></td>
<td>Mean 4</td>
<td>64.1</td>
<td>2.6</td>
<td>1.4</td>
<td>50.7</td>
<td>7.1</td>
<td>43.6</td>
<td>0.2</td>
<td>6.9</td>
<td>41.3</td>
<td>8.5</td>
</tr>
</tbody>
</table>
Nitrate load increased with increasing streamflow (Figure 2.3). The seasonal variation in nitrate was well reproduced in the calibration period, except for some overestimation occurring mainly during December 2003, when precipitation was higher than in other months and reached 143 mm. The nitrate loadings during the validation period showed patterns similar to those in the calibration period.

During the wettest months in 2006, slight overpredictions were found. The exception was in November 2006, when very high precipitation totaling 237 mm was registered and the nitrate load was underestimated (Figure 2.3). The driest months fitted well in terms of both the range and the dynamics of nitrate. The $\xi_{NS}$ and $R^2$ values for monthly nitrate load were 0.65 and 0.76, respectively. Seasonal variations in nitrate concentrations in streams displayed a seasonal pattern over the studied period. The concentrations ranged from 1 to 16 mg N L$^{-1}$ and averaged 6.9 ±2.7 mg N L$^{-1}$ in late spring and in summer, and the highest nitrate values ranged from 3.10 to 16.5 mg N L$^{-1}$ and averaged 7.4±3.2 mg N L$^{-1}$ in winter from January to February (Figure 2.4). The simulation of monthly nitrate concentrations by the SWAT model was, however, rather poor when compared with measured data (Figure 2.4), although the simulation of increasing and peak nitrate concentrations was reasonably fair, the modelling of decreasing and low nitrate levels in streamflow seems to be prone to high uncertainty and errors. This can be partly explained by the relatively poor representation of riparian stream areas by the SWAT model.

The highest stream nitrate exports were observed for subcatchments 1, 4, 9, 12 and 13 (Figure 2.5), with winter wheat, maize, alfalfa and other intensive agricultural land use (i.e. sunflower and tomato) as prevailing crops.
Figure 2.4. Measured and simulated nitrate concentration values (mg N L\(^{-1}\)) in stream waters from 2003 to 2008.

There was a significant positive correlation (R = 0.5, n = 13) between stream nitrate export by streamflow and total agricultural area in each sub-catchment (p < 0.05), as shown in Figure 2.5. In this study, the SWAT model predicted inputs and outputs to evaluate the overall nitrate budget components. Major inputs were fertilizers and wet deposition by rainfall and biological N fixation (Table 2.5). The total basin-averaged inputs, which include fertilizers (75 kg N ha\(^{-1}\) yr\(^{-1}\)), biological fixation (1.4 kg N ha\(^{-1}\) yr\(^{-1}\)) and deposition (2.6 kg N ha\(^{-1}\) yr\(^{-1}\)), reached 80 kg N ha\(^{-1}\) yr\(^{-1}\) annually and thus are clearly dominated by agricultural practices in the catchment (Table 2.5).

With regard to outputs, nitrate leaching was the most important output, representing 55 kg N ha\(^{-1}\) yr\(^{-1}\) loading of the shallow aquifer (Table 2.5). We also examined an irrigation scenario (Table 2.5) using shallow groundwater as water source, and considering a percentage irrigated area of 3.93% or 1380 ha of maize and alfalfa. The model predicted a slight increase in total catchment evapotranspiration of 7.8 mm (245.5 mm versus 237.7 mm). For the irrigated
crop area itself, the increase in evapotranspiration amounted to 198 mm, from irrigation volumes of 286 mm received by the pivot systems (see also Table 2.1).

Figure 2.5. Stream nitrate export by streamflow versus crop land area by sub-basin ($r = 0.5$, $p < 0.05$). Numbers in the figure correspond to each sub-basin.

Due to the limited irrigated area (3.93%), annual average catchment irrigation volume remains rather low at 11.2 mm. Basin water yield showed a slight decrease (262.7 mm versus 265.1 mm), owing to a lower shallow aquifer baseflow (Table 2.5). The SWAT model predictions of the annual basin area-averaged nitrate budget (Table 2.5) indicated a slight increase in values for N fertilizer amount (80.2 kg N ha$^{-1}$ yr$^{-1}$), with no changes for wet deposition by rainfall (2.6 kg N ha$^{-1}$ yr$^{-1}$) and biological N fixation (1.4 kg N ha$^{-1}$ yr$^{-1}$). In terms of nitrate outputs, the annual basin area-averaged nitrate budget showed small increases in values for shallow aquifer leaching (55.4 kg N ha$^{-1}$ yr$^{-1}$) and uptake by plants (45.6 kg N ha$^{-1}$ yr$^{-1}$), when compared to the no-irrigation scenario.
2.4 Discussion

2.4.1 Streamflow prediction and nitrate load

The streamflow of the Roxo catchment was successfully estimated by the SWAT 2005 model for an eight-year simulation period (2001–2008). The model predictions generally performed well for a monthly time step during the calibration and validation periods and were concordant with guidelines established for monthly simulations (Moriasi et al., 2007). Coefficient of determination ($R^2$) and Nash–Sutcliffe simulation efficiency ($\xi_{NS}$) values (Table 2.4) supported the model predictions. We obtained a strong relationship between observed and predicted streamflow. However, slight overpredictions of streamflow at the end of the summers of 2001, 2003 and 2006 (September–October) were recorded. This is probably due to overemphasis on the direct runoff component versus delayed runoff (Bosch, 2008). Mean monthly streamflow showed a seasonal decreasing pattern, following a classic pattern for drier climatic areas (Molenat et al., 2007), with several smaller streams drying up during the summer (June–September). Components of the mean annual water balance (Table 2.5) showed that baseline hydrological calibration yielded mean annual values for direct surface runoff of 49 mm and baseflow of 216 mm. The baseflow fraction was found to be 81% of the total annual basin water yield of 265 mm. In accordance with our study ($\xi_{NS} = 0.60; R^2 = 0.77$), similar nitrate studies using the SWAT model for small streams and reservoirs in the United States showed comparable efficiencies ($\xi_{NS} = 0.65; R^2 = 0.68$) for validation (Bosch, 2008). Chu et al. (2004) obtained $\xi_{NS}$ values of 0.52 in a small agricultural catchment in Maryland, and Chaplot et al. (2004) predicted mean monthly nitrate loads in the Walnut Creek watershed (51.3 km²) in Iowa with a determination coefficient of 0.73. Nitrate load was overestimated strongly in November 2006 (a month with extreme excess rainfall), and this can be attributed mostly to the overestimation of streamflow. Moreover, when high rainfall occurs in the autumn and winter months, high nitrate levels in streamflow are noted, owing to important contributions from aquifer nitrate outflow. However, these $\text{NO}_3^-$ concentrations in the baseflow stream may be overestimated, because the SWAT model does not account well for biogeochemical processes (e.g. denitrification) in the shallow aquifers, which naturally reduce nitrate levels in groundwater. It is also possible that the simulated nitrate load peak (9 kg N ha$^{-1}$) during the wettest months can be
attributed to a simplification of shallow aquifer and baseflow processes such as nitrogen transformations and water flows. De Vos et al. (2000) found that higher nitrate values in the water table during winter were associated with the water flow and nitrate transport processes such as mineralization and denitrification. The highest nitrate loads (14 kg N ha⁻¹) were observed in the rainy season (November 2006), which can be attributed to the very high rainfall and runoff occurring during that month (Figure 2.2). However, during the entire period relatively high nitrate concentrations reaching 16.5 mg N L⁻¹ were recorded (Figure 2.3). Therefore, water flowpaths, such as the soil to shallow groundwater to stream pathway, might play an important role in determining nitrate levels in catchment runoff. The SWAT model predicted that most of the nitrate loadings to the streams would originate from baseflow, which was confirmed by the presence of a high nitrate concentration (11.6 ± 1.28 mg N L⁻¹) measured in wells during different samplings in 2003, 2005 and 2008 (Figure 2.6) and other research (Paralta, 2001).

![Figure 2.6. Nitrate concentration in shallow groundwater (wells) during different sampling (June 2003, September 2005 and October 2008).](image-url)
The nitrate loadings during the validation and calibration periods showed similar patterns. During the extremely wet month of November 2006, nitrate load was underestimated, whereas in the drier years (2004 and 2005) of the simulation period some slight over-predictions are visible (Figure 2.3). Besides higher runoffs, the higher nitrate loads recorded in autumn can also be attributed to the increase in nitrogen mineralization during these months, which can be explained by warm ambient temperatures and relatively high soil moisture content. The drier months fitted well in terms of both the range and the dynamics of nitrate. The $\xi_{NS}$ and $R^2$ values for monthly nitrate load were 0.65 and 0.76, respectively. Duarte et al. (2008) also used SWAT to simulate streamflow and nitrate loads in the Rio Formosa watershed located in the southern part of Portugal, using different periods for calibration and validation, and they obtained variations similar to those reported in this study. Nitrate export occurred mostly in rainy periods, and also with higher concentrations in baseflow. The high nitrate concentration in baseflow seems to be the result of increased drainage from a shallow fractured layer (Gabbros de Beja) present in the Piooses sub-catchment area in the northern part of the catchment (Paralta, 2001). Regarding the irrigation scenario for maize and alfalfa (3.93% of catchment area), the annual water balance components changed only slightly or remained the same (Table 2.5). This result is due to the relatively limited size of the total irrigation area, which is small in proportion to the total catchment area. The potential evapotranspiration, obtained using the Penman–Monteith method, computed by SWAT and totalling 1328 mm for both irrigation and no-irrigation scenarios, is in agreement with previous recorded data and evaluations (1237–1376 mm) for the area (Paralta, 2001). A slight increase (7.2 mm) in actual evapotranspiration in the catchment water balance when irrigation is applied is rather obvious.

The relatively low value is again due to the limited proportion of irrigated land in the catchment. Because irrigation and crop evapotranspiration are at the expense of shallow groundwater, a small decrease (~3.3 mm) in baseflow and basin water yield is noted, although the SWAT model predicted a slight (+0.8 mm) increase in direct surface runoff. Regarding the nitrate budget components, small increments in nitrate leaching and nitrate uptake by plants were found in the irrigation scenario (Table 2.5). The small difference in the components
corresponds to the combined effects of fertilization and increased soil moisture content and water fluxes owing to irrigation.

### 2.4.2 Seasonal changes in nitrate export

The highest monthly nitrate losses were systematically recorded in autumn and winter, especially in November–December 2003 and December 2006 (Figure 2.3). This can be explained mainly by nitrate accumulation in soils during drier periods (spring–summer), which is later mobilized and transported by higher rainfall, soil moisture, runoff and baseflow generation to the streams. Seasonal patterns of nitrate losses with significantly lower values during summer have been reported for agricultural catchments (Gao et al., 2004; Arheimer and Liden, 2000). One explanation for lower nitrate loss during this period might be the presence of stagnating waters in the catchment stream network during spring and summer periods, which in the case of high temperatures and low levels of oxygen are favorable for denitrification and act as nitrogen sinks in catchments. Another explanation is the increased plant uptake and removal by periphyton and plants (Flipo et al., 2007). Further, with the near absence of rainfall and very low streamflow in summer, much less nitrate is transported to the small streams in the catchment. Nearly 80% of the annual export of nitrate occurs from October to February. These results are comparable to those of an eight-year study by Beaudoin et al. (2005), which reported annual loads associated with amounts of drainage and flow in an agricultural catchment located in the north of France.

### 2.4.3 Land use and nitrate exports

In general, nitrate export is significantly related to the presence of local N sources, which vary according to land use distribution in the catchment. Sub-catchments dominated by agricultural fertilized crops such as maize, wheat and alfalfa exported five times more nitrate than sub-catchments covered by forest and range. In contrast, low nitrate export from forested sub-catchments is not surprising, because forests have high nitrogen retention capacity as they are subject to repeated biomass removal (Hayakawa et al., 2006). The agricultural sub-catchments (1, 4, 9, 12 and 13), where the highest export was registered (Figure 2.5), have small ponds, mainly for irrigation purposes, which suggests that local aquatic environments also play a significant part in the processes controlling nitrate losses in the Roxo catchment. Monthly nitrate exports from
agricultural catchments in Europe and the United States are similar to those found in our studied (Table 2.5) catchment (David et al., 1997; Bechmann et al., 1998; Filoso et al., 2003; Beaudoin et al., 2005; Isidoro et al., 2006).

2.4.4 Nitrate budget

The SWAT model offers the possibility of simulating the hydrological and chemical behaviour of catchments and enables the overall nitrate budget to be quantified and evaluated. Only a few studies have conducted this analysis (i.e. Bosch, 2008; Hu et al., 2007). Our SWAT predictions enabled us to assess the biogeochemical transport in the Roxo catchment and to explain the causes and magnitude of nitrate fluxes. We identified the annual basin area averaged nitrate budget and the main contributions of nitrate to the basin outflow (Table 2.5). Major nitrate contributions to streamflow in the Roxo catchment originate from the use of fertilizer on maize, winter wheat, alfalfa and some minor crops. Maize typically receives more fertilizer than other crops, around 150–200 kg N ha$^{-1}$. Symbiotic biological N fixation by Rhizobium bacteria can produce tens of kilograms of nitrogen per hectare per year, but is limited to only a few species of leguminous crops of economic importance (Olivares, 2008). Therefore, N fixation is considered an important input in agricultural fields where alfalfa is produced, and also contributes to nitrate exports to the streams and ultimately to the main reservoir. Basin-averaged N fixation (1.4 kg N ha$^{-1}$ yr$^{-1}$) remains relatively low owing to the limited area of atmospheric N-fixing crops in the basin. However, high values have been found for alfalfa in studies in Alentejo, Portugal, where up to 100 kg N ha$^{-1}$ yr$^{-1}$ can be fixed (Ferreira et al., 2005). Our total N fixation value (1.4 kg N ha$^{-1}$ yr$^{-1}$) is lower than the predicted inputs commonly found in semiarid and agricultural fields such those found (4 kg N ha$^{-1}$ yr$^{-1}$) in Southern Spain (González de Molina et al., 2010).

Although our results showed that the main inputs for the whole catchment were fertilizers, biological fixation was more important in some subcatchments (1, 2, 6 and 13), apparently being an important source of nitrogen in some of the catchment areas. The high nitrate inputs into the catchment have certainly influenced all components of the biogeochemical nitrogen cycle. These inputs commonly transform the ecosystem by high nitrogen export, increment of nitrate in groundwater, and increase of the denitrification process. With regard to the outputs, most of the nitrate leaving the soil system is leached to the shallow aquifer and subsequently reappears as baseflow to the stream network.
and reservoir. According to the model, leaching to the shallow aquifer represented 75% of the soil nitrate losses in the catchment, amounting to 55 kg N ha\(^{-1}\) yr\(^{-1}\). From this amount, 14% percolated to the deep aquifer and 86% or 47 kg N ha\(^{-1}\) yr\(^{-1}\) reappeared as baseflow nitrate in the catchment streamflow (Table 2.5). Results were compared and validated with nitrate concentrations (11.6±1.28 mg N L\(^{-1}\)) from shallow groundwater from municipal and private wells, sampled during 2003, 2005 and 2008 (Figure 2.6). Plant nitrogen uptake is also important (43 kg N ha\(^{-1}\) yr\(^{-1}\)), but only a fraction of this (19%) is removed by crop harvest operations (Table 2.5). The basin-averaged nitrate removal by harvest operations was 8.9 kg N ha\(^{-1}\) yr\(^{-1}\). In loamy soils in an agricultural field in the north of France, Beaudoin et al. (2005) found nitrate leaching values between 11 kg N ha\(^{-1}\) and 42 kg N ha\(^{-1}\). This is in agreement with the values for most of the Luvisol and Vertisol soils in our catchment. It confirms that leaching is related to soil and crop types and farmer practices (Hall et al., 2001). In Luvisol in Elvas (South Portugal), Carranca et al. (1999) observed that an important part of N from fertilizer was lost by leaching, especially in autumn and winter. Hence, excess fertilizer application and nitrate leaching can be seen as the most important sources and pathway of increased nitrate loading in streams, with strong evidence of high nitrate leaching in the wettest periods (Carranca et al., 1999; Boyer et al., 2002; Boyer et al., 2006). It would be beneficial to decrease the use of fertilizers during the autumn and winter periods in order to reduce nitrate leaching. Stream nitrate, however, was also strongly correlated with total runoff, reflecting the high mobility of this anion in general. With regard to the denitrification processes, these were estimated to account for 11% of the total nitrate output of the catchment, with 7.1 kg N ha\(^{-1}\) yr\(^{-1}\) as basin area-weighted average. The highest values are found in sub-catchments 5 and 10, with 15.5 and 17 kg N ha\(^{-1}\) yr\(^{-1}\), respectively, where several large ponds are present. These higher values correspond to denitrification rates found in hotspots in riparian areas surrounding water reservoirs in Eastern China (Wang et al., 2010, 2009). It is common to find that the small streams in the area are not active and are almost dry during spring and summer, with only the presence of local stagnant water spots. Certainly, these small ponds act as real riparian hotspots or buffers, with low oxygen values (<5.5 mg L\(^{-1}\)), temperatures reaching 35 °C in summer, high dissolved organic carbon values (>8 mg L\(^{-1}\)), high levels of sulphate (>125 mg L\(^{-1}\)), and high levels of nitrate (16 mg N L\(^{-1}\)) in these stagnant waters in streambeds (Yevenes
and Mannaerts, 2012). If we combine these factors, we suspect that denitrification is a likely N output in the stream network. Limited literature indicates that denitrification in small seasonal and ephemeral streams is a seasonally important sink for nitrate before it reaches larger permanent streams and impoundments such as reservoirs and lakes. Lehmann et al. (2003) reported that during a stagnation period microbial nitrate reduction takes place in the stream water column when low oxygen conditions are present. Gentry et al. (2009) showed in-stream denitrification to be substantial during summer time.

The SWAT model losses by denitrification for the upper Roxo basin averaged 7.1 kg N ha$^{-1}$ yr$^{-1}$. In our experimental measurements of potential denitrification in soils, we found the total mean denitrification rate in the Roxo catchment soils to be 3.9±2.9 kg N ha$^{-1}$ yr$^{-1}$ (Gamises, 2009; unpublished data), which is to some extent in agreement with the model. Cheshire et al. (1999) reported that NO$_3^-$ loss in soils around Beja city, inside the Roxo catchment, could be attributed to denitrification. In general, growing-season denitrification is not desirable on most agricultural and forested land because the denitrifiers are competing with plants for inorganic N. Molenat et al. (2007) pointed out that denitrification cannot be used to explain the NO$_3^-$ decrease in a system when the conditions required for denitrification have neither been met nor become evident. We need a significant decrease in the soil or sediment redox potential and available organic carbon or pyrite, the most common electron donors for heterotrophic denitrification to take place. Overall, our catchment nitrate budget component analysis indicates a slight positive N balance for the three scenarios (Table 2.5). This excess probably indicates over-fertilization, as the reduction scenario points to a lower N balance excess value. However, the uncertainties and simplifications in the biogeochemical processes simulated with the SWAT model do not permit us to draw more conclusions than an examination of the overall N balance. In summary, our SWAT simulations enabled us to determine that excess fertilizer application is causing rapid (seasonal) nitrate leaching, probably by direct solute leaching (owing to limited microbial immobilization or lack of plant uptake) and increments in the mineralization in the Roxo catchment. These processes can contribute to increasing soil acidity and diminishing soil fertility, as well as to impacts on water bodies through eutrophication, which nowadays is regularly observed and recorded in the main reservoir (Chisha, 2003; Gurung, 2005; EMAS, 2008).
2.4.5 Fertilizer scheme scenarios

During the last 40 years, the studied region has yielded 75% of the country’s total wheat production (Paralta and Oliveira, 2005), but the amounts of fertilizer that are applied every year are not exactly known. Therefore, the cumulative chemical export to streams and reservoir impoundments so far is also unclear. Finally, there is little doubt that \( \text{NO}_3^- \) leaching and subsequent reappearance in the baseflow is a major source of nitrate in surface waters, contributing to local water quality problems and the nutrient loading of the Roxo reservoir. A model-based analysis was carried out to assess best fertilizer management practices, with the intention of analysing the impacts of a 20% reduction in current nitrogen fertilizer application rates. This percentage was in agreement with values used for the same agricultural crop conditions in similar catchments. The proportion chosen is also in accordance with European (EU) specifications within the framework of the Common Agricultural Policy (Council Regulation No. 2078/92/EEC), which advocates the adoption of environment-friendly farming practices. In order to protect water resources, farmers have to reduce the nitrogen fertilization level by 20% relative to the optimum level and establish catch crops before all spring crops. Furthermore, Bracmort (2010) suggested that a 20% reduction in nitrogen-based fertilizer from baseline applications of 10% and 20% is also useful in nitrous oxide (\( \text{N}_2\text{O} \)) mitigation alternatives for agricultural soil management. Model outputs showed that a 20% fertilizer reduction can considerably decrease the \( \text{NO}_3^- \) exports (Table 2.5). Similar predictions for maize fields with a 20% fertilizer reduction have been reported for the United States by Jaynes et al. (2001), showing approximately 28% less \( \text{NO}_3^- \) export from maize fields. However, because most of the agricultural land in the Roxo catchment has been over-fertilized during the last 40 years, we cannot expect significant differences in the main crop yields and nitrate leaching in response to a reduction in fertilization. The soil nitrogen pools (organic and inorganic N) are high, and will change only gradually after a reduction in fertilizer N inputs in the soil system. It is also possible that the mineralization of the soil organic N was underestimated or the leaching or denitrification was overestimated, which reduced the nitrate before the plant uptake. Despite the fertilizer N rate reduction, the model predicted a small excess of nitrate, indicating some accumulation of N in the soil. One might expect that a fertilizer deficiency in crops would lead to a reduction in soil N (Jaynes et al., 2001).
2.5 Conclusion

This study of stream nitrate losses in the upper Roxo reservoir catchment was conducted using the SWAT model and eight years of observed weather, hydrological, chemical soil and water quality data. In periods with low leaching losses and minimal denitrification, nitrate is accumulated and carried over to the next year, thus partly offsetting the net depletion of soil N. Agricultural practices and seasonal fluctuations were the main reasons for high temporal variations in nitrate exports via small streams to the main reservoir. Our study suggests that seasonal fluctuations and winter wheat and maize agriculture play an important role in the variations in the nitrate losses via the stream network to the Roxo reservoir. In general, our catchment nitrate budget analysis indicates that N fertilizers are the largest inputs, but biological fixation and wet deposition by rainfall can also be important N sources and contribute to the catchment nitrogen budget. Nitrate export from the agricultural lands occurred mainly through soil leaching to the shallow aquifer and resulting baseflow pathway. The results of this study help us to quantify and understand the seasonal and land use impacts on nitrate loading patterns in the catchment. In the Roxo catchment, N fertilizer reduction schemes can be evaluated as possible control strategies, in terms of adjustment to the requirements of the European Nitrate and Water Framework Directives.
Chapter 3

Water and nitrate sources by chemical appraisal in a streams of a reservoir catchment
Abstract
The knowledge of water source contributions (stream water, alluvial sediment pore water, precipitation and shallow groundwater) were analysed using diagnostic tools and end-member mixing analysis (EMMA) in the main streams of Upper Roxo catchment, Portugal. We evaluated whether a limited number of spatially distributed geochemical tracer data ($\delta^2$H, $\delta^{18}$O, Cl$^-$, SO$_4^{2-}$, Na$^+$, NO$_3^-$ and K$^+$) sampled were sufficient to quantify water flow pathways and nitrate sources in the streams. Results showed that, when only wet season data were modelled, streamflow chemistry was controlled and generated by three end-members: shallow groundwater, alluvial sediment pore water and precipitation. Isotope signatures of stream water were located mostly below the local meteoric water line (LMWL) and plotted along a local evaporation line (LEL), reflecting the permanence in the streamflow of shallow groundwater subjected to prior evaporation. Measured and historical stream nitrate concentrations appeared to be strongly related to shallow groundwater. The results of this study have improved our understanding of water source contributions to streamflow in the catchment, and also yielded indications of nitrate consumption related to biogeochemical processes in the streamflow. Moreover, we could conclude that the relatively limited geochemical spatial sample database used in this study was an adequate input for the end-member mixing analysis and diagnostic tools to quantify water sources and nitrate origins in the streamflow of the catchment.

3.1 Introduction

Identification of flow pathways and mechanisms for streamflow generation is necessary in order to better understand the interactions between land and aquatic systems in catchments (Uhlenbrook et al., 2008). Characterizing nutrient transports in water environments is an equally important challenge because of the multiple options and pathways that a nutrient might follow in a watershed (Mulholland and Hill, 1997). Many studies have been carried out regarding the role of in-stream processes as decisive factors of stream water chemistry. Such processes can be strongly related to hydrological conditions such as connectivity among streams, temperature regime, rainfall occurrence and intense evaporation (Dunn et al., 2006; Liu et al., 2008a; Meredith et al., 2009). Recent studies have linked hydrological and geochemical aspects in order to better understand the role of water pathways in nutrient transport by streamflow (McHale et al., 2002; Bernal et al., 2006; Ocampo et al., 2006; Tesoreiro et al., 2009).

To identify streamflow components and nutrient releases at the catchment scale, water isotope approaches in conjunction with geochemical tracers have regained importance (Bernal et al., 2006; Mul et al., 2008; Liu et al., 2008b; Meredith et al., 2009; Hrachowitz et al., 2010). Hence the use of conservative chemical tracers is again increasing rapidly conducting hydrograph separation and exploring streamflow origins and hydrological or geochemical processes occurring in the aquatic environments of watersheds (Ocampo et al., 2006; Didszun and Uhlenbrook, 2008).

An effective analytical tool to help recognize the importance of various streamflow components is the use of endmember mixing analysis (EMMA) through principal components analysis (PCA), developed by Christophersen and Hooper (1992). A water sample taken from different water origins or hydrological flow paths such as precipitation, runoff or streamflow, sediment or soil pore water, and shallow or deep groundwater represents a mixture of water that contributes to generating the streamflow. If these distinct water flow paths are considered as end-members assumed to be conservative and constant over time, it is possible to define a system of simple linear equations for calculating the mixing proportions between water flow paths. When more than two tracers are considered, EMMA can be used, with reliable results, to identify end-
member values and to help to build a conceptual understanding of the streamflow generation process.

The possibility of analysing water hydrochemistry on a continuous basis and for longer time periods in larger catchments requires a very significant budget and considerable time and effort, and was beyond the scope of this research. Therefore, the aims of this study were to see whether a spatial sample-based analysis, using several seasonal observation campaigns, could be used as a data source for conducting end-member mixing analysis, using isotope and hydrochemical signatures to decipher the water and dissolved chemical (nitrate) origins in the streamflow of a catchment and the inflow in a reservoir.

3.2 Materials and Methods

3.2.1 Site Description

The stream network (20 km²) is composed of three intermittent streams: Chaminé-Pisoses, located in the northern section of the catchment; Juliana, located in the middle part; and Louriçais, located in the southern branch of the streams. All these small streams flow into the main reservoir of the catchment (Figure 3.1). The streamflow varies strongly depending on the season. It is common to find low flow connectivity in the streams during summer periods, especially from July to September.

The municipal waters from Beja city are channelled through sewers to a waste water treatment plant, and effluent is released into the Chaminé-Pisoses catchment tributary. Hydrogeological investigations have revealed that the main productive aquifers of the upper Roxo catchment are located in the Beja-Acebuches and Beja Gabbro geotectonic complexes. These geological formations consist mainly of gabbro-dioritic rocks. The altered bedrock varies locally and can reach 30m in thickness. It creates an unconfined aquifer with a shallow water table, which drains naturally into the Roxo stream network (Paralta and Oliveira, 2005). The main drainage runs from the north and north-east to the south-west and into the reservoir. The other catchment drainage originates in the south-west and drains to the north-east to finally reach the reservoir (Figure 3.1).
3.2.2 Water sampling and analysis

For this study, 27 sampling sites were chosen in the stream network. The sampling points were divided over three larger streams covering the north, middle and south of the catchment (Figure 3.1). In total, 87 water samples (stream water, sediment pore water and shallow groundwater from wells) were collected during three periods. Sampling in wet conditions was conducted in two periods: the first during of autumn in October 2008 and the second during late winter in March and April 2009. Sampling in dry conditions took place from August to September 2009.

To avoid contact with the atmosphere, stream water, shallow groundwater and sediment porewater samples were taken in duplicate, using a vacuum pump technique. Pore water samples were collected by inserting a rhizon sampler syringe (Seeberg–Elverfeldt et al., 2005) into drill holes in a core 52 cm long located in the bottom of the stream (P1, P2 and P3) near to the sites A3, A4 and A5. Samples were stored in 10-ml glass vials. Ten rhizon samplers were then inserted horizontally into the core at depths of 0, 1, 2, 3, 5, 7, 10 and 20 cm below the sediment-water interface. For this study, we considered only four depths (0, 5, 10 and 20).

Figure 3.1. Map of study area of Roxo catchment with the principal pollution sources. Circles are the 27 sampling sites, including stream water (A), shallow groundwater (W) and pore water samples (P).
To measure several physicochemical parameters (Cl\(^-\), SO\(_4^{2-}\), Na\(^+\), NO\(_3^-\) and K\(^+\)) and stable isotopes (δ\(^2\)H and δ\(^18\)O), samples were filtered in situ using Millipore 0.45 μm filter pore size and a vacuum pump. After filtration, all samples were immediately stored at 4°C in a dark environment for subsequent chemical and isotopic analyses.

Shallow groundwater samples were taken at depths ranging from 2 to 5 m from private and municipal wells at several locations. This water table is considered representative of the natural drainage of the groundwater aquifer towards the stream network and ultimately the Roxo reservoir.

Anion concentrations were analysed using a Hach UV-Vis spectrometer at the Faculty of Geosciences of Utrecht University. Precipitation chemistry data were extracted from the Global Atmosphere Watch (GAWSIS). Isotope signatures for δ\(^2\)H and δ\(^18\)O were determined by isotope ratio mass spectrometry in the Environmental Isotope Laboratory in Vienna, a facility certified by the International Atomic Energy Agency (IAEA). Isotope results are expressed in deviations from the Vienna Standard Mean Ocean Water (VSMOW) standard per mil, using the usual delta notation. Stable isotopes in precipitation were obtained from the IAEA and the Global Network of Isotopes in Precipitation (GNIP), using measured data for Beja city, which is located in the upper northeast of the catchment.

3.2.3 Hydrological data

Daily records of rainfall data (2008-2009) were obtained from automatic weather stations located near Beja and Aljustrel. In addition, a 2008-2009 dataset of daily evapotranspiration, reservoir storage volume, historical reservoir water levels and water abstraction data was available from ABROXO (2009). An inverted reservoir water balance method was used to estimate the total catchment streamflow into the main reservoir water body. This mass balance technique consisted of estimating the reservoir inflow from the variation over time in the reservoir storage volume and the total sum of outflows from the reservoir. This method proved to give reliable estimates of

1 http://www.wmo.int/pages/prog/arep/gaw/gaw_home_en.html
2 http://www-naweb.iaea.org/napc/ih/IHS_resources_gnip.html
catchment total streamflow and inflow in the reservoir (Vithanage, 2009). Streamflow was also measured at sampling locations in the catchment during the three sampling campaigns, using the chemical dilution technique (Herschy, 1995).

3.2.4 Isotopic framework

The conventional isotope hydrology concept introduced by Craig (1961) uses the relationship between $\delta^2$H and $\delta^{18}$O concentrations in natural waters from different places in the world and compares isotopic enrichment relative to ocean water. This relationship shows a linear correlation over the entire range of waters that have not undergone excessive evaporation and is defined by the global meteoric water line (GMWL). The GMWL was generated from isotope data of rivers, reservoirs and precipitation from various countries, and is defined by the best-fit line $\delta^2$H = 8$\delta^{18}$O + 10. Monthly precipitation samples of any region give rise to a local meteoric water line (LMWL) and, together with the relationship to the GMWL, are useful in explaining the relationship between the water and respective hydrological processes (Karim and Veizer, 2002). The LMWL is useful for interpreting local water movements, sources, and processes to which the water has been subjected (Dansgaard, 1964).

Our isotopic framework was based on the interpretation of local evaporation lines (LEL) as described by Wolfe et al. (2007). The LEL is useful for determining the evaporation processes that have occurred in the various water sources. It is defined by a regression line through isotopic compositions of evaporating water surfaces in the catchment such as shallow groundwater.

3.2.5 End-member mixing analysis (EMMA)

Contributions of different end-members to streamflow were determined using geochemical tracers based on EMMA in combination with diagnostic tools of mixing models. In brief, EMMA (Christophersen and Hooper, 1992) entails a mixing model for identifying potential water flow paths (end-members) and their proportions that contribute to streamflow. EMMA embraces a principal component analysis (PCA), a commonly applied technique that is used to reduce the dimensionality of a multivariate database. The aims of PCA were i) to find a lower dimensional space (PCA space or U space) in which the stream water is found and, ii) to describe the variability of the data. The dimensionality
of the PCA space is determined by the number of principal components or eigenvectors obtained from the PCA using Eq. (3.1).

\[ \mathbf{U} = \mathbf{X}^* \mathbf{V}^T \]  

(3.1)

where \( \mathbf{U} \) represents an \((n \times m)\) matrix of stream chemical data consisting of \(n\) samples and \(m\) one less than the number of end-members. \( \mathbf{X}^* \) represents an \((n \times p)\) matrix, where \(p\) represents the geochemical tracers. \( \mathbf{V} \) has a dimension \((m \times p)\).

Christophersen and Hooper (1992) proposed that the number and identification of the potential end-members could be determined by plotting the end-members in the PCA mixing space defined by stream water and defining the end-members represented in the stream water. More recently, Hooper (2003) suggested that the number (or rank) of the end-members could also be determined from stream chemistry data, using only diagnostic tools.

Diagnostic tools of mixing models can be used to determine the geochemical tracers and the number of end-members in streams (Liu et al., 2008a). Stream data are used to develop a correlation matrix, followed by PCA to determine eigenvectors. The standardized stream data are projected into \( \mathbf{U} \) space by multiplying the data by the eigenvectors, and then the PCA residuals are computed using Eq. (3.2).

\[ \tilde{\mathbf{X}}^* = \mathbf{X}^* \mathbf{V}_1^T (\mathbf{V}_1 \mathbf{V}_1^T)^{-1} \mathbf{V}_1 \]  

(3.2)

where \( \tilde{\mathbf{X}}^* \) is the standardized stream data and \( \mathbf{X}^* \) is the projection of the standardized data using eigenvectors of \( \mathbf{V}_1 \). \( \mathbf{V}_1 \) was extracted using a correlation matrix of the stream data.

If the residuals show a random pattern in a 1D eigenvector and a high \( p \) probability is found, then this means two end-members are needed (Hooper, 2003). The aim of using this combination of diagnostic tools instead of a simple mixing model is to reduce the uncertainty in choosing the number of end-members and selecting conservative geochemical tracers.

We generated four scenarios with solutes (\( \text{Cl}^- \), \( \text{SO}_4^{2-} \), \( \text{Na}^+ \), \( \text{NO}_3^- \) and \( \text{K}^+ \)) and two stable water isotopes (\( \delta^{2}H \) and \( \delta^{18}O \)) obtained from 87 samples of stream
water, sediment pore water and shallow groundwater from wells, and additional chemistry data of precipitation in the 352 km² catchment during 2008-2009.

(1) Using the streams dataset in Matlab (R2011b version 7.13.0.564), we applied diagnostic tools such as PCA residual analysis and p probability to estimate the number of end-members from stream water and the conservative tracers to be used (Hooper, 2003). Residual analysis was used to examine the variability of water chemistry (difference between predicted and observed tracer concentrations), which was plotted against the observed sample. A random pattern of residuals indicates a conservative mixing subspace, while a structure in the residuals can be attributed to non-conservative behaviour or poor selection of end-members (Hooper, 2003).

(2) End-member mixing analysis was then used with geochemical tracers determined earlier to identify end-members and to quantify the contributions of end-members to streamflow. The identification of end-members was evaluated using the distance between the original chemical compositions and U-space projections (PCA scores). The first U-space projection was used to select the end-members and examine whether the projections of end-members were different from streamflow.

(3) The validation of end-member contributions (shallow groundwater, pore water and precipitation) was calculated with the distance shown as a percentage, and by dividing distance by the original chemical composition. The shorter distance the better the fit of an end-member to EMMA. This methodology is mathematically comparable to a common mixing model, for example hydrograph separation using one tracer for two components whereas we used the first U-space projection (U₁) in a two-end-member solution (Liu et al., 2008a).

3.2.6 Spearman’s rank correlation

Once the end-member mixing analysis had been performed, we used a non-parametric Spearman’s rank correlation test to investigate whether there was a relation between stream nitrate concentration (mg N L⁻¹) and the percentage contribution of end-members or water sources to the streamflow.
3.3 Results

3.2.7 Streamflow and rainfall for 2008-2009

Streamflow in the catchment shows a strong seasonal fluctuation, with several smaller creeks of the stream network having an intermittent flow regime. Figure 3.2 illustrates the precipitation and streamflow response of the catchment, using a 10-day (decade) time interval. Lowest precipitation was recorded during spring and summer, typical of dry Mediterranean areas. Major rainfall events were registered during winter, particularly at the end of January 2009, reaching 54.6 and 44.3 mm. Stream flows were generally low, around 0.05 m$^3$ s$^{-1}$ during summer months, mainly in August and September 2009, while the highest 10-day time-averaged value reached 1.89 m$^3$ s$^{-1}$ at the beginning of February 2009.

Figure 3.2. Decade (10-day) time interval for precipitation and streamflow of Roxo catchment during the study period (October 2008 to September 2009).
3.3.1 Spatial variation of stable water isotopes

The stable water isotope composition (expressed as δ-values) revealed systematic differences in the streams and wells, especially during September 2009 (Table 3.1). For the entire period (2008-2009), minimum (-24.8 and -3.92) and maximum (28.5 and 8.78) composition (δ2H and δ18O) values were recorded in streams. Isotopic measurements of δ2H and δ18O in shallow groundwater from wells showed a mean of -16 ‰ and -2.58 ‰ and -11.9‰ and -1.17‰, respectively (VSMOW scale). Isotopic signatures of historical Beja’s station precipitation data (Paralta et al., 2007) has been typically located near to the global meteoric water line (GMWL), showing a calculated LMWL of δ2H = (7.6 ± 1.2) δ18O + (8.3 ± 9.1) on Figure 3.3a. Most of the stream water sample points were located below the LMWL although some were approaching the LMWL. The LEL is shown along the regression line (δ2H = 4.6δ18O + 1.5), with a coefficient of determination (R²) of 0.9 (Figure 3.3b). The LEL connects the samples from stream water, shallow groundwater and the Roxo reservoir to precipitation samples. Low water flow is shown to be enriched in the heavy isotopes in streams and shallow groundwater, and typically more depleted in the case of precipitation. Regarding the δ* symbol shown in the LEL plot (Figure 3.3b), it corresponds to the isotopic composition of mean weighted local precipitation and refers to water input from precipitation.
<table>
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<th></th>
<th>October 08</th>
<th>March 09</th>
<th>September 09</th>
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<tr>
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<tr>
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<td>1.40</td>
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<tr>
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<td>15</td>
</tr>
<tr>
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<tr>
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<tr>
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<td>st. dev.</td>
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<tr>
<td>pore water</td>
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<tr>
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<td>17.0</td>
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<tr>
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<tr>
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<tr>
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<td>0.34</td>
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<tr>
<td>N</td>
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</table>

Table 3.1 Descriptive statistics of measured values in the stream water, wells and pore water.
3.3.2 Hydrochemistry

Figure 3.4a, 3.4b and 3.4c indicate the field measurements for $\delta^2$H, $\delta^{18}$O, Cl$^-$, K$^+$, Na$^+$, NO$_3^-$ and SO$_4^{2-}$ in the streamflow, shallow groundwater and sediment pore water, respectively. Solute concentrations in streamflow did not vary significantly over the three sample periods, while spatial variation was always presented (Figure 3.4a).

Sites corresponding to A1, A8-A17 showed important peaks in stable isotopes and solute concentrations. Higher chloride and sulphate concentrations were detected in the streamflow at site A1, which is close to the outfall of a municipal waste water treatment plant (Figure 3.4a). Figure 3.4c relating to sediment pore water showed a clear trend of increasing nitrate concentration nearer to the surface water. In addition, nitrate concentration in the top of the sediment is much larger than the concentration in the surface water.

3.3.3 Geochemical tracers and numbers of end-members

Scenario A1: We included all the data from wet and dry seasons, with five solutes as geochemical tracers (Cl$^-$, K$^+$, Na$^+$, NO$_3^-$ and SO$_4^{2-}$) of sediment pore water, shallow groundwater and precipitation samples. PCA residual analysis using five solutes was highly structured against measured concentrations in streamflow in the 1D mixing space, with $R^2$ usually higher than 0.3 and $p=0.07$. 
The degree of randomness significantly increased in the 2D mixing space, but only for \(\text{Cl}^-\) and \(\text{SO}_4^{2-}\). The residuals in the 2D mixing space were, however, still correlated with streamflow solute concentrations for most tracers, with \(R^2 > 0.3\). Therefore, there was no random pattern between predicted and observed values, and the well-structured residual distribution indicated that there was no conservative mixing in this scenario. Hence, using dry and wet sampling data indicated a non-conservative behaviour and also no conservative mixing of end-members. Therefore, only one single end-member is considered in the streams. Further EMMA was therefore not relevant with regard to this dataset.

Scenario A2: We included all the data from Scenario A1 and we added stable isotope data (\(\delta^2\text{H}\) and \(\delta^{18}\text{O}\)) of shallow groundwater and precipitation.
Figure 3.4. Chemical composition of $\delta^{2}H$, $\delta^{18}O$, $\text{Cl}^{-}$, $\text{SO}_4^{2-}$, $\text{Na}^+$, $\text{NO}_3^-$ and $\text{K}^+$ in: a) surface water (squares the Chaminé stream to the reservoir, inverted triangles indicate the reservoir to Victoria stream, b) shallow groundwater and c) pore water during the three periods. Sampling points (A1 to A17) are numbered from 1 to 17 in the plots.
Residuals from all solutes and isotopes were also well structured against measured concentrations in streamflow in the 1D mixing space, with $R^2$ usually higher than 0.4 and $p=0.08$. The residuals in the 2D mixing space were also correlated with streamflow solute concentrations for most solutes, with $R^2$ near 0.6 and $p<0.01$. In conclusion, this scenario using dry and wet season data also indicated a non-conservative behaviour and no conservative mixing of end-members. Hence, only one single end-member was found in the streams. We therefore split the datasets and removed the dry season samples from the analysis.

Scenario B1: We used only the wet season samples gathered during October 2008 and March 2009 and we used five solutes as geochemical tracers ($Cl^-$, $K^+$, $Na^+$, $NO_3^-$ and $SO_4^{2-}$) of streamflow. Figure 3.5 shows the distributions of residuals of $Cl^-$, $Na^+$and $SO_4^{2-}$ in a random pattern in the 1D mixing space, with $R^2$ values less than 0.2 and $p=0.4$. In contrast, $NO_3^-$ showed a highly structured pattern, with $R^2$ of 0.90 and $p<0.001$, and $K^+$ with $R^2$ of 0.42 and $p=0.03$ and which was not enough for it to be taken into account in the next analysis. The variability increased in the 2D mixing space, with $R^2$ less than 0.1 and $p>0.2$. Therefore, according to this variability in the first and secondary components, the streamflow chemistry was controlled by the three solutes $Cl^-$, $Na^+$and $SO_4^{2-}$ and defined by conservative 2D mixing space, which means three end-members and can give us signals of flowpath proportional contributions.

Therefore, a new PCA was made using only $Cl^-$, $Na^+$ and $SO_4^{2-}$, which are conservative upon mixing, and these solutes were employed in EMMA using the three end-members (sediment pore water, shallow groundwater and precipitation). PCA scores as U-space projections were calculated using the eigenvectors extracted from these conservative tracers.

Scenario B2: We used the data from scenario A1 and available stable isotopes ($\delta^2H$ and $\delta^{18}O$) of streamflow. Figure 3.6 shows the distributions of residuals between original concentrations, and predicted values for the 1D and 2D mixing spaces referred to the main two principal components. This figure shows that the distributions of residuals of $Cl^-$, $SO_4^{2-}$, $\delta^2H$ and $\delta^{18}O$ show a near-random pattern in the 1D mixing space, with $R^2$ values less than 0.2 and $p>0.3$. The
variability increased in the 2D mixing space for the four tracers, with $R^2$ less than 0.1 and $p > 0.4$. In contrast, in this scenario $\text{Na}^+$, $\text{NO}_3^-$ and $\text{K}^+$ presented more structured values for $R^2$ of 0.25, 0.96 and 0.54 and $p < 0.1$, respectively, which was not enough to take them into account in the next analysis. Therefore, according to this variability the streamflow chemistry was primarily controlled by conservative 2D mixing space, and then three end-members can give us signals of flowpath proportional contributions. The geochemical tracers ($\text{Cl}^-$, $\text{SO}_4^{2-}$, $\delta^2\text{H}$ and $\delta^{18}\text{O}$) detected with the residual analysis were used to run a new PCA for the studied period, EMMA and streamflow separation. Two eigenvectors were adequate (indicating three potential end-members) for shallow groundwater, pore water and precipitation.
Figure 3.5. Plot of residuals versus original concentrations of solutes and stable isotopes for Scenario B1 (outliers are identified in the plot).
Figure 3.6. Plot of residuals versus original concentrations of solutes and stable isotopes for Scenario B2 (outliers are identified in the plot).
A closer look at the residual plots from Scenarios B1 and B2 (Figure 3.5 and 3.6) permitted two outliers to be detected (originating in the two field campaigns from sample point A1 and A9). Confrontation with field observations and evidence indicated point source contamination from a waste water treatment plant outfall (A1) releasing residual waste water directly into the streams. This waste outfall typically increases salt concentrations as Cl\(^-\) and SO\(_4^{2-}\) as well as nitrogen levels i.e. ammonia and organic-N, but not nitrate concentrations as shown in Figure 3.5 and 3.6. The PCA and residual analysis therefore proved to be good diagnostic tools for the detection of contamination as well. The practical use of outliers in diagnostic analysis and EMMA was also suggested by Hooper (2003).

3.3.4 Identification and validation of end-members

A new PCA was made for Scenarios B1 and B2, considering the three potential end-members and with only the geochemical tracers that had passed the residual analysis: Cl\(^-\), SO\(_4^{2-}\) and Na\(^+\) for Scenario B1 and \(\delta^{2}H\), \(\delta^{18}O\), Cl\(^-\), SO\(_4^{2-}\) for B2. PCA scores as U-space projections were calculated using the eigenvectors extracted from these geochemical tracers using the correlation matrix indicated in section 3.2.5. The difference between the first (U\(_1\)) and secondary (U\(_2\)) U space was projected for the appraisal of end-members. U\(_1\) and U\(_2\) values of shallow groundwater samples were slightly higher than those of streamflow and similar to those of pore water.

Finally, end-members were identified using the orthogonal distance between their original compositions and U-space projections from the PCA. For Scenario B1, a hydrograph separation permitted the proportional contributions from the end-members to be derived: groundwater 52%, sediment pore water 38% and precipitation 10%. For Scenario B2, this data scenario analysis led to the following proportional contributions from the end-members: groundwater 56%, sediment pore water 32% and precipitation 12% − which is similar to Scenario B1.

3.3.5 Sources of nitrate

Once we had obtained the estimated water source contributions through EMMA, we then correlated the end-member contributions with the stream nitrate concentrations for the wet season. Here we used a Spearman’s rank
correlation test to assess relations between stream nitrate concentration and the percentage of streamflow for the main end-members. Shallow groundwater and sediment pore water percentages showed a good correlation with stream nitrate values ($\rho_{\text{wells}}$ and $\rho_{\text{porewater}}$ 0.56 and 0.79, respectively). Apparently pore water is playing an important role in the exchange of nitrate with the stream water. An additional Spearman’s rank correlation test using historical nitrate data of June 2003 and December 2004 from shallow groundwater and stream water yielded similar results ($\rho_{\text{wells}}$: 0.52 and 0.65) and confirmed our hypothesis on nitrate source origins (Table 3.2). Moreover, if we compared our highest $\text{Cl}^-$ concentrations in stream water and shallow groundwater, the concentrations of chloride showed synchronicity between concentrations of streamflow and shallow groundwater. This implies that chloride concentrations in the stream depend upon the relative contribution from shallow groundwater, where $\text{Cl}^-$ concentrations are more stable than in the stream waters.

Table 3.2. Historical nitrate levels and the Spearman's Rho coefficient between measured nitrate in wells and the proportion of water from the space projections of EMMA.

<table>
<thead>
<tr>
<th>Date</th>
<th>Water sample</th>
<th>$\text{NO}_3^-$ range</th>
<th>Spearman rank ($\rho$)</th>
<th>N</th>
<th>Reference</th>
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<tr>
<td>Jun-03</td>
<td>streams</td>
<td>0.78-16.6</td>
<td>-</td>
<td>25</td>
<td>Historical campaign from Water Resources Department*</td>
</tr>
<tr>
<td></td>
<td>wells</td>
<td>3.5-17.7</td>
<td>0.52</td>
<td>25</td>
<td></td>
</tr>
<tr>
<td>Dec-04</td>
<td>streams</td>
<td>0.29-12</td>
<td>-</td>
<td>2</td>
<td>Paralta et al., 2007</td>
</tr>
<tr>
<td></td>
<td>wells</td>
<td>0.61-14.2</td>
<td>0.65</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>May-06</td>
<td>streams</td>
<td>-</td>
<td>-</td>
<td></td>
<td>Paralta et al., 2007</td>
</tr>
<tr>
<td></td>
<td>wells</td>
<td>7.9-33</td>
<td>-</td>
<td>11</td>
<td></td>
</tr>
<tr>
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<td>wells</td>
<td>1.7-20.2</td>
<td>0.49</td>
<td>10</td>
<td>Our study</td>
</tr>
<tr>
<td>Mar-09</td>
<td>wells</td>
<td>2.2-25.6</td>
<td>0.56</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

### 3.4 Discussion

#### 3.4.1 Water isotopes and solutes abundance in Roxo catchment

Over the entire study period, a low streamflow regime prevailed and isotope signatures were below the LMWL and close to, but slightly different from, the LEL, reflecting a mixture of shallow groundwater and a small influence of rainfall sources (Figure 3.3a and 3.3b). The precipitation values define the
LMWL, which is only marginally above the GMWL (see Figure 3.3a). The slight deviation of the LMWL from the GMWL is a confirmatory point, since such behaviour of precipitation composition has been commonly observed in similar drier regions (Meredith et al., 2009). The data from the LEL (Figure 3.3b) provided information on the secondary processes acting on the water as it travels from its source into the surface water. However, the LEL for stream water and shallow groundwater, $\delta^2H = 4.6\delta^{18}O + 1.5$ (Figure 3.3b), indicated that these waters have experienced evaporation. These enriched or higher $\delta$-values for $\delta^2H$ and $\delta^{18}O$ isotopes can be observed during September 2009 (Table 3.1). Stream waters ($8.78^{o/oo}$) were enriched in heavy isotopes as a consequence of evaporation with respect to shallow groundwater (from $-3.42^{o/oo}$ to $2.16^{o/oo}$). It is known that during low-flow periods in semi-arid areas evaporation generates characteristically heavy isotope enrichment in residual surface waters above $+3^{o/oo}$ (Gonfiantini, 1986). The difference in stable isotope composition between the shallow groundwater and the stream water can be used for tracing the contributions of water to the streams. This is hinted in Figure 3.3b, where shallow groundwater samples are not that close to the stream samples in summer sampling in the LEL. This fractional contribution is confirmed by the EMMA in the next sections.

Heavier isotopic values were also related to higher anion concentrations. Chloride and sulphate, as suitable indicators of the concentration of salt in the water due to their conservative nature showed high concentrations (Table 3.1). In general, $\text{Cl}^-$ and $\text{SO}_4^{2-}$ concentrations were largest in the streams, ranging from 21.9 to 891 mg L$^{-1}$ and from 13.3 to 313 mg L$^{-1}$, respectively. These values are due mainly to the shallow depth and intermittent pools in several sections in the stream network that permit water stagnation and evaporation. Major values were observed in the first sampling point (A1; corresponding to the stream location downstream of the waste water treatment plant outfall) (Figure 3.1 and 3.4a). It is known that NaCl contributions from households can thoroughly change water quality (Appelo and Postma, 2007). High chloride and sulphate concentrations in wells showed high correlation ($R=0.97$), indicating an evaporative mechanism in combination with a longer residence time. Outliers corresponding to site A1 were identified using the residual plots from Scenarios B1 and B2 (Figure 3.5 and 3.6). This indicated either errors in the data or different processes controlling the chemistry of the water samples.
3.4.2 Evaluation of end-member mixing analysis

The numbers of end-members and conservative geochemical tracers were determined by combining chemical and isotope data from stream water, based on Christopher and Hooper (1992) and Hooper (2003). The selection of end-members and number of conservative tracers was quantitatively evaluated using diagnostic tools of mixing models as residual analysis from PCA (Figure 3.5 and 3.6). Two scenarios for the wet season campaign with different combinations of solutes and isotopes were well reproduced (Figure 3.5 and 3.6).

We agree that the seasonal campaigns using approximately 30 points for hydrochemistry and isotopes and additional information on continuous streamflow can be criticized for using a small sample size in time. However, this data analysis permitted us to verify to what extent relatively limited spatial samples and a few discrete but well-chosen sample periods could indicate source contributions of water and nitrate to a catchment streams.

3.4.3 Biogeochemical control

Hydrological flow paths have significant functions in controlling catchment-scale biogeochemical processes. Contributions of water flow paths identified using geochemical tracers can be used to distinguish hydrological and biogeochemical control of nitrogen (Mulholland and Hill, 1997). Therefore, nitrate levels in streamflow can be predicted using several flow components determined by geochemical tracers, and then compared with measured values. Over- and under-estimation of nitrate concentrations in stream water suggest gain or loss of nitrate during streamflow generation, and thus identify the dominant processes controlling this nutrient (Mulholland, 2004). In our study, shallow groundwater is the main contributor to the streamflow and shows a positive relation with stream nitrate concentrations. However, indirect evidence of the importance of in-stream processes also comes from the analysis, showing that nitrate concentrations in the streams are lower than in the shallow groundwater. This difference cannot be explained by a simple mixing of water coming from the small tributaries to the main stream. It can be most probably attributed to nitrate consumption such as denitrification in the stream water or streambed. This can be demonstrated for instance by lower (<5 mg L⁻¹) dissolved oxygen concentrations (DO) in the streams (Table 3.1). However, it
would be necessary to quantify the in-stream assimilation, nitrification or denitrification processes in the stream water and sediment.

Preliminary experimental analysis and the results of this study in sediment pore water also suggest that in-stream processes are occurring in the streambed. Pore water values show that nitrate concentrations decreased below the interface stream water-sediment and later slightly increased with depth, which is likely involving biogeochemical reactions (Figure 3.4c). As well, conservative solutes such as Cl- varied only slightly with depth in the sediment. One explanation of the low nitrate concentration at the water-sediment interface is the nitrate consumption and the microbial activity at the interface and in the top layer of the sediment (Curie et al., 2009). The limited length (20 cm) of the sediment core permitted us to identify the biogeochemical interactions at the sediment water interface, but not the full pathway towards the groundwater (Pfenning and McMahon, 1997).

The higher nutrient concentrations in some points of the streams appear to come from the localized organic-rich materials and sediments transported by superficial slope runoff and deposited naturally near the stream network. In addition, the presence of livestock, such as herds of cattle, sheep breeding or goats and intensive pig farming (Figure 3.1) that use the stream network as a source of drinking water, may induce additional fertilization and local differences in stream chemistry.

### 3.5 Conclusion

This study of the intermittent stream network of the upper Roxo catchment in South Portugal involved three seasonal spatial sampling campaigns and quantitative hydrochemical and isotope analysis. Our objective was to see whether a spatial sample-based analysis, using seasonal observation campaigns, could be used as a data source for conducting end-member mixing analysis to identify water and nitrate sources to the streamflow. The non-parametric Spearman’s rank analysis was used to correlate source contributions to nitrate levels in the streamflow. End-member mixing analysis in combination with exploratory analysis was used to identify and quantify the proportional contributions of the main water sources to the streamflow. The analysis used
geochemical tracer data from precipitation, stream water, alluvial sediment pore water and shallow groundwater.

The use of a relatively limited number (~30 points) of spatially distributed sampling points in one dry and two wet seasons permitted us to distinguish shallow groundwater as the major contributor to streamflow in all analysis periods. Proportional contributions in the two scenarios were around 50% for groundwater, followed by sediment pore water (~40%) and rainfall (~10%). The largest hydrological source contributor, shallow groundwater, appears also directly related to stream nitrate concentrations. A high porewater proportion in the streamflow means that it contributes significantly to the control of the water chemistry of the streams. A relation between stream nitrate and precipitation and also direct surface runoff could not be derived from the datasets. The analysis suggests that the main nitrate pathway to the stream network in this catchment is through soil leaching and re-appearance in the shallow aquifer baseflow. This biogeochemical aspect, together with the other nitrogen cycle components in the catchment, is currently being investigated and will be reported in another research contribution. Further studies would also be necessary in order to elucidate stream nitrate responses to extreme storm events.
Water and nitrate sources
Chapter 4

Investigating stream denitrification using isotopic analysis and 1-D reactive transport modelling in an agricultural catchment.
Abstract

Agriculturally impacted streams are important sites for nitrogen removal by processes such as denitrification. Knowledge of the spatial patterns and the different factors affecting this process can help to identify hotspot areas. In this context, stable nitrate isotopes ($\delta^{15}$N$_{\text{NO}_3^{-}}$ and $\delta^{18}$O$_{\text{NO}_3^{-}}$) and a 1-D reactive transport model were used to study the processes that lead to nitrogen transformation and losses in small shallow streams that flow into a reservoir. The streams are located in an intensively cultivated reservoir catchment (352 km$^2$) in the Alentejo region, South Portugal. Water sampling and analysis was seasonal (October 2008, March 2009 and September 2009) and consisted of 17 stream and reservoir water samples, 10 shallow groundwater well samples and three stream sediment pore water samples. Historical streamflow and water quality data of 2003, 2008 and 2009 were used to support the implementation of a 1-D reactive transport model. High nitrification rates resulted in high nitrate concentrations in the upstream part and in the shallow groundwater in the northern part of the catchment. This was followed by nitrate removal leading to low nitrate levels downstream and in the reservoir. Denitrification could be detected using the $^{15}$N isotopic signature of nitrate in the streams but not in the reservoir waters where very low nitrate levels were measured. In the streams, the calculated isotopic enrichment factor for nitrate was $-2.9\%$, from which we derive that denitrification accounted for 7 to 22 % of the nitrate removal. This study contributes as one of the first coupled studies that include identification of nitrification and denitrification using isotope analysis in combination with 1D reactive transport modelling in streams of a Southern European meso-scale catchment.

This chapter is based on:
4.1 Introduction

Recent studies suggest that the nitrogen cycle is changing more rapidly than any other biogeochemical cycle and the excessive introduction of reactive nitrogen in the environment is the third global harmful problem, after biodiversity loss and climate change (Gilles, 2005; UNEP, 2007). One of the major scientific challenges concerning this excess reactive nitrogen is to better understand nitrogen release mechanisms to the atmosphere and the implication of nitrogen removal within terrestrial and aquatic ecosystems (www.denitrification.org, GANE 2005; Groffman et al., 2009; NinE, 2010; NinE-ENA, 2010).

Agricultural streams receive large amounts of reactive nitrogen (i.e. nitrate, ammonium, organic nitrogen) from land and transfer this to streams, lakes, reservoirs and ultimately oceans (Mulholland, 1992). Nitrate is highly soluble and denitrification enables nitrate to be eliminated from streams. Some studies suggest that shallow agricultural streams might be hotspots for the denitrification process, due to their more rapid uptake and transformation of inorganic dissolved nitrogen than higher streams (Peterson et al., 2001, Groffman et al., 2009, Alexander et al., 2009). These biogeochemical processes appear to be highly efficient over relatively short distances in streams, emphasizing that shallow streams are not simply nutrient conduits, but also zones of important biogeochemical transformations (Ashkenas et al., 2004).

Denitrifying bacteria reduce nitrate ($\text{NO}_3^-$) via nitrite ($\text{NO}_2^-$), nitric oxide (NO) and nitrous oxide ($\text{N}_2\text{O}$) to the non-reactive dinitrogen gas ($\text{N}_2$) commonly with organic carbon as electron donor (Devol, 2008). Denitrification is considered a particularly challenging process to measure and model (Groffman et al., 2009). Much of this challenge arises from the fact that small sites (hotspots) frequently account for a high percentage of the denitrification activity, which is regulated by environmental factors such as substrate availability, temperature, and oxygen concentration in aquatic ecosystems.

Stable isotope signatures of dissolved nitrate are powerful tools to study denitrification processes and the origin of nitrate inputs because dissolved nitrate sources such as wastewater and fertilizers and denitrification tend to have distinct isotopic signatures (Lake et al., 2001; Vander Zanden et al., 2005; Diebel and Vander Zanden, 2009). During denitrification, as nitrate levels
decrease, residual nitrate becomes enriched in heavy isotopes $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ as a result of natural isotopic fractionation (Kendal and McDonnell, 1998, Pintar et al., 2008). It is well known that denitrification results in isotopic changes in the nitrate pool, as bacteria preferentially reduce $\delta^{14}\text{N}_{\text{NO}_3^-}$ over $\delta^{15}\text{N}_{\text{NO}_3^-}$, leaving an enriched pool of $\delta^{15}\text{N}_{\text{NO}_3^-}$ (Mariotti et al., 1984b, Søvik and Mørkveck, 2008).

Interpretation of nitrate data to identify biogeochemical processes such as denitrification in streams has been done using different modelling analyses (Chen et al., 2004; Ocampo et al., 2006; Böhlke et al., 2009; Wexler et al., 2011). However, only few studies have combined numerical simulation of reactive transport of nitrate with stable isotopes (Hill et al., 2000, Chen et al., 2004; Beaulieu et al., 2011). Here we present the results of a 1-D reactive transport model in a stream at the catchment scale. The model solves nitrate and ammonium and includes two biogeochemical pathways, nitrate removal and nitrification. Nitrate removal rates are compared with denitrification rates as estimated from the nitrate isotopic signature. The aim of this combined approach is to determine the transport and fate of reactive nitrate and its spatial behaviour along a hydrological flow gradient.

4.2 Materials and Methods

4.2.1 Water sampling

A map of the streams and reservoir indicating positions of the sampling points is shown in Figure 3.1. Samples were collected midstream from 17 stream sites (A1 to A11 from upstream to reservoir inlet in the main Chaminé river) and water from 10 shallow wells with water table levels between 2 and 5 m. Also sediment pore water was sampled at 4 sites. The sampling campaigns were conducted in summer and winter respectively in October 2008, March 2009 and September 2009. All water samples were collected by a peristaltic water pump connected to a water collector and filtered in the field at 0.45 μm. Samples were stored in non-reactive plastic bottles with double seal caps. Physical and chemical parameters were analysed in situ in the field (Cl$^-$, SO$_4^{2-}$, NO$_3^-$, HCO$_3^-$, Temperature (°C), Electrical conductivity (EC), and pH).
Chapter 4

Stream sediment porewater profiles were sampled with minimum disturbance by inserting a rhizon sampler syringe (Seeberg-Elverfeldt et al., 2005) into drill holes in a core 52 cm long located in the bottom of the stream pools (P1, P2 and P3) near to sites A3, A4 and A6. Ten rhizon samplers were then inserted horizontally into the core at depths of 0, 0.5, 1, 2, 3, 5, 7, 10, 15 and 20 cm below the sediment-water interface. Samples were stored directly in 10-ml glass vials without contact to the atmosphere. Nitrate concentrations were analysed in the laboratory.

Cation and anion concentrations were analysed by inductively coupled plasma-optical emission spectrometry (ICP-OES) and Atomic Absorption Spectrometry (AAS) at ITC/University of Twente and ion chromatography (HPLC) at the Faculty of Geosciences of the Utrecht University. Water samples collected for isotopic composition (δ¹⁵N₅O₃⁻ and δ¹⁸O₅O₃⁻) were analysed through the bacterial denitrification method and determined by isotope ratio mass spectrometry, in the certified ISOFS-Environmental Isotope laboratory of Ghent University.

4.2.2 Stable isotope calculations

The isotopic results in this study are expressed using the delta notation (δ), which is the parts per thousand difference in the ratio of the less abundant isotope to the most abundant isotope, relative to the same ratio in a reference standard (Vienna Standard Mean Ocean Water for Oxygen-18 and an open atmosphere air standard for Nitrogen-15), and defined by:

\[
\delta^{15}N = 1000 \frac{R_{sample} - R_{air}}{R_{air}}; \quad \delta^{18}O = 1000 \frac{R_{sample} - R_{smow}}{R_{smow}}
\]

(4.1)

where \( R_{sample} \) and \( R_{air} \) and \( R_{smow} \) are the isotopic ratios (\( \delta^{15}N/\delta^{14}N \) or \( \delta^{18}O/\delta^{16}O \)) for the sample and for the reference standard for \(^{15}\text{N}\) and \(^{18}\text{O}\), respectively. Stable isotopes of \( \text{NO}_3^- \) are useful for assessing sources and for distinguishing between \( \text{NO}_3^- \) sinks (Ruehl et al., 2007).

Partitioning of isotopes between two compounds containing the same element with different isotopic ratio is called isotope fractionation (Mariotti et al., 1981) and can be defined through the kinetic fractionation factor as follow:
Stream denitrification using isotopic analysis and 1D reactive transport modelling

\[ \alpha_{p-s} = \frac{R_p}{R_r} \]  

(4.2)

Where \( R_p \) and \( R_r \) are heavy to light isotope (\(^{15}\)N/\(^{14}\)N ratios) in the product (residual nitrate) and reactant (or substrate), respectively (Kendal and McDonnell 1998). Biologically mediated denitrification enriches residual NO\(_3^-\) in both \( \delta^{15}\)N\(_{NO_3^-}\) and \( \delta^{18}\)O\(_{NO_3^-}\), whereas other NO\(_3^-\) sinks result in little or no enrichment. Therefore, the magnitude of \( \delta^{15}\)N\(_{NO_3^-}\) enrichment associated with nitrate removal is quantified through an enrichment factor. The kinetic fractionation factor is typically described in terms of an enrichment factor (\( \varepsilon \)) as follows:

\[ \varepsilon = (\alpha - 1)1000 = \frac{(\delta_p - \delta_r)}{(\delta_r + 1000)}1000 \]  

(4.3)

Where \( \delta_p \) and \( \delta_r \) are the delta values of the product and the reactant, respectively (Sharp 2007). The enrichment factor is negative for most of the nitrogen transformation processes, and in the case of denitrification it has been defined with a range from -40 to -3.5‰ which reveal a variety of environmental and experimental conditions (Lund et al., 2000; Kendal and McDonnell, 1998, Böttcher et al., 1990, Mariotti 1984b, Lehmann et al., 2003).

The enrichment factor (\( \varepsilon \)) also can be estimated as:

\[ \varepsilon = \frac{\delta^{15}N - \delta^{15}Ni}{\ln(\text{NO}_3^-) - \ln(\text{NO}_3^-_i)} \]  

(4.4)

where \( \delta^{15}\)N is the stable isotope ratio for NO\(_3^-\), and \( \delta^{15}\)Ni and NO\(_3^-_i\) are the N stable-isotope ratio and concentration, respectively, of NO\(_3^-\) prior to N removal (or the initial value). Processes that leave residual NO\(_3^-\) enriched in \( \delta^{15}\)N\(_{NO_3^-}\) and \( \delta^{18}\)O\(_{NO_3^-}\) have negative enrichment factors because \( \delta^{15}\)N tend to increase as NO\(_3^-\) decreases.

The evolution of the isotopic composition of the residual reactant (Mariotti et al., 1981) can be defined as:

\[ \delta_R = \delta_{R_0} + \varepsilon \ln\left(\frac{\text{NO}_3^-}{\text{NO}_3^-_i}\right) \]  

(4.5)
where $\delta_R$ is the $\delta^{15}\text{N}\text{NO}_3^-$ or $\delta^{18}\text{O}\text{NO}_3^-$ value of the reactant nitrate at time $t$, $\delta_{R_0}$ is the initial $\delta^{15}\text{N}\text{NO}_3^-$ or $\delta^{18}\text{O}\text{NO}_3^-$ value of the nitrate, $(\text{NO}_3^-/\text{NO}_3^-i)$ is the remaining fraction of nitrate, and $\varepsilon$ is the enrichment factor (Kendall and McDonnell, 1998). Enrichment factors for $\delta^{15}\text{N}\text{NO}_3^-$ and $\delta^{18}\text{O}\text{NO}_3^-$ in surface waters were determined by regression of $\delta^{15}\text{N}\text{NO}_3^-$ or $\delta^{18}\text{O}\text{NO}_3^-$ on ln$(\text{NO}_3^-/\text{NO}_3^-i)$.

Finally, the $\delta_R$ values are transformed to percentage (%) to get the degree of denitrification for each sampling point (Chen et al., 2009). To calculate the degree of denitrification from our data, the initial isotopic composition $(\delta_{R_0})$ was chosen by the lowest $\delta^{15}\text{N}\text{NO}_3^-$ value during the samplings. The initial value $(\text{NO}_3^-i)$ for nitrate concentration corresponded to the same point as the initial isotopic composition.

### 4.2.3 Numerical Approach

To assess the dynamics of dissolved inorganic nitrogen (i.e NO$_3^-$ and NH$_4^+$) in the water, we developed a 1-D reactive transport model for NO$_3^-$ and NH$_4^+$ for the main stream (Chaminé) of the catchment (approx. length 15 km). The one dimensional reactive transport model was developed in the open source software R (R Development Core Team, 2009; http://www.r-project.org/). The R package ReacTran (Soetaert and Meysman, 2012) permits to use the volumetric advective-diffusive transport function in R (eq. 4.6):

$$\frac{\partial C}{\partial t} = -\frac{1}{A_x} \cdot \frac{\partial (A_x \cdot C)}{\partial x} + \frac{1}{A_x} \cdot \frac{\partial}{\partial x} \left( A_x \cdot \frac{\partial C}{\partial x} \right) + \text{reaction} \quad (4.6)$$

where $t$ is time, and $x$ is distance along the stream axis; the first term represents transport by the stream flow (advection) and the second term represents (turbulent) dispersion. The assumption is made that the cross-sectional area ($A_x$) is constant in time (Alexander et al., 2009), but it varies along the stream axis ($x$). The chemical state variables in the reactive advection dispersion model, $C$, were NO$_3^-$ and NH$_4^+$, and are described in terms of concentration (mg N L$^{-1}$). The reactions comprise two main biogeochemical processes: nitrate removal (denitrification) and nitrification. Boundary conditions for nitrate and ammonium were derived from stream campaigns; nitrification and denitrification rates from literature were used.
In order to simulate the nitrate dynamics in the streamflow, only the main stream of the catchment was modelled. The Chaminé tributary connects a main stream (upstream boundary) to the reservoir (downstream boundary). Steady state flow conditions \(Q\) representative for the sampling period were assumed in the model, to be able to focus on the biogeochemical reactions and transformations of nitrate in the streamflow. The cross-sectional area \(A_x\) was estimated from the surface areas at the sampling points and linearly interpolating in between. The length axis was defined such that the stream boundary is located at 0, the downstream reservoir boundary is at 15 km. We consider the mean streamflow being the annual long term (2003, 2008 and 2009) averages (Vithanage, 2009).

The major reactions are nitrification and denitrification as given by following biochemical reactions:

\[
\begin{align*}
\text{NH}_4^+ + 2\text{O}_2 & \rightarrow \text{NO}_3^- + 2\text{H}^+ + \text{H}_2\text{O} \\
5\text{CH}_2\text{O} + 4\text{NO}_3^- & \rightarrow 2\text{N}_2 + 4\text{HCO}_3^- + \text{CO}_2 + 3\text{H}_2\text{O}
\end{align*}
\]

Both the nitrification rate \(k_1\) and the denitrification rate \(k_2\) was modelled as first order to the reactant:

\[
k_1 = r_1 \cdot \text{NH}_4^+ \\
k_2 = r_2 \cdot \text{NO}_3^-
\]

For nitrification and denitrification.

To maintain the model as simple as possible and due to the lack of data to constrain anammox rates, we assumed nitrification of stream ammonium to be the only source of nitrate and denitrification to be the only N-loss term. Thus, we did not include exchange across the water-sediment interface (Hoffman et al., 2008).

### 4.2.4 Biogeochemical databases and boundary conditions

Chemical data from 2003, 2005 and 2008 were obtained by different fieldwork campaigns from ITC, as well as from the Sistema Nacional de Informação de Recursos Hídricos (SNIRH), Empresa Municipal de agua e saneamento de Beja.
(EMAS-Beja). Our sampling data from 2008 and 2009 were added to the dataset. For the modelling, the solute concentrations from the first most upstream sampling point (A1) were considered as upstream boundary conditions for the stream and sampling point (A11) from the reservoir was used as the downstream boundary condition.

Daily rainfall data from 2008 until 2009 was obtained from automatic weather stations located nearby Beja and Aljustrel area (Figure 3.1). Stream flow was measured in the sampling sites when the water level was enough to allow measuring it. Furthermore, daily streamflow were obtained using precipitation, evapotranspiration and reservoir storage volume, and water use databases, using an inverted reservoir water balance approach. This procedure is based on the estimation of the reservoir inflow from the variation over time of the storage volume of the reservoir and the total outflow from the reservoir (Vithanage, 2009; Yevenes and Mannaerts, 2011). Water depths were usually very low (0.1 to 0.5 m) in the streams and was measured during the fieldwork campaigns. For each year, a constant, average flow value was used in order to obtain and simulate a steady state system for flow.

4.2.5 Modelling calibration and boundary conditions
Model calibration and verification consisted on testing whether the designed model was able to reproduce qualitatively and quantitatively the observation data (Soetaert and Herman, 1995). During this model evaluation step we confronted model predicted outputs with observation data. Data from the years 2009 were used to calibrate, while the data from 2003 and 2008 were used to verify the model.

4.3 Results
4.3.1 Solutes and isotopes in streams and wells
Values of physical and chemical parameters, measured during the three field campaigns in the streams are summarized in Table 4.1. The streamflow regime is characterized by slow flows (average 0.1 to 0.2 m/s) and shallow flow depths ranging from 0.05 to 0.5 m, depending on the local stream cross section shape. Average long term annual flow (2003-2009) was 0.10 m$^3$s$^{-1}$, with seasonal low values and the end of summer of 0.04 m$^3$s$^{-1}$. High daily flow peaks are typically
recorded at the end of winter and reached values of 1.89 m³ s⁻¹ as in February 2009 during our study campaign. Stream water temperature averaged 21 ± 8 °C during the entire period and electrical conductivity varied in the seasons with values ranging from 783 μS cm⁻¹ in March 2009 to 3100 μS cm⁻¹ for September 2009. Stream water showed slightly basic pH values with an average value of 8.66 ± 0.88. Dissolved oxygen levels in October 2008 showed an average value of 6.0 ± 1.7 mg O₂ L⁻¹ with oxygen increasing significantly in downstream direction towards the reservoir (Figure 4.1). Oxygen values increased during winter (5.5-11 mg O₂ L⁻¹) when also streamflow is higher.

In the shallow groundwater wells, during the winter sampling, means of T°C, EC, and pH, corresponding to 15.8 ± 1.5°C, 850 ± 113  μS cm⁻¹ and 7.9 ± 0.39 were lower than summer with averages of 22.7± 1.99°C, 1290 ± 896 μS cm⁻¹ and 9.23 ± 0.73, reflecting longer residence time in summer than in winter.

![Figure 4.1. Oxygen levels in the main stream Chaminé river (upstream) to the Roxo reservoir (downstream) of the three campaigns. Vertical bars indicate standard deviation. Sampling points are A1 to A12, as depicted in Figure 3.1](image)

Oxygen content showed a wide range in winter ranging from 2.18 to 10.4 mg L⁻¹ in contrast with summer when values varied between 4.5 and 8.3 mg L⁻¹. Nitrate concentrations ranged from 0.01 to 16.5 mg N L⁻¹, with mean values of
7.24 ± 5.45 in October 2008, 5.70 ± 5.49 during March 2009 and 3.36 ± 3.57 mg N L$^{-1}$ during September 2009 (Table 4.1). The highest NO$_3$ concentration was registered in the second (A2) and third (A3) stations (Figure 4.2a) after which they decreased through the Chaminé stream towards Roxo reservoir, reaching nitrate values below the detection limit < 0.1 mg N L$^{-1}$ (Figure 4.2a). The same decreasing trend in river nitrate was observed for the southern subcatchment tributary (Figure 4.2c).

![Figure 4.2. a) Nitrate concentration, nitrogen and oxygen isotopes in the Chamine stream. Averages of the three campaigns: vertical bars indicate standard deviation. b) Average of the Degree of denitrification for every site. Sampling points area A1 to A11, as depicted in Figure 3.1.](image)

Figure 4.3. Nitrate concentration in the Louriçais river, Roxo catchment. Sampling points area A12 to A17, as depicted in Figure 3.1.

![Figure 4.3. Nitrate concentration in the Louriçais river, Roxo catchment. Sampling points area A12 to A17, as depicted in Figure 3.1.](image)

Nitrate concentrations in the reservoir were always significantly lower than in the upper catchment streams. During the wetter sampling period (March 2009),
nitrate in the streams were highest reaching 15 mg N L⁻¹. While in the reservoir in the same period, nitrate was almost not detectable. Nitrate concentrations in wells were generally higher than in streams ranging 0.1 to 25.5 mg N L⁻¹. The highest mean were registered in March with 9.8 ± 8.6 mg N L⁻¹ and the lowest in September with 6.79 ± 8.4 mg N L⁻¹ (Table 4.1).

Figure 4.4. Linear regression of $\delta^{15}N_{NO_3}^{-}$ on ln $(NO_3^-)$, where ln $(NO_3^-)$, is the fraction of the original pool of nitrate still present. The slope of the regression is equal to the isotopic enrichment factor $\varepsilon$ (here −2.9‰) for denitrification in the streams.

Isotopic composition of Nitrogen ($\delta^{15}N_{NO_3}^{-}$) ranged from 3.02‰ to 15.1‰ averaging 7.40‰. The oxygen isotope ($\delta^{18}O_{NO_3}^{-}$) ranged from 4.29‰ to 30.9‰ and averaged 11.3‰. Systematically, nitrate isotopic values were always below detection limit in the reservoir points and southern part of the catchment (Table 4.1, Figure 4.2c), due to the systematic low nitrate concentrations observed at these locations. In general, as the stream water flows from the upstream source to the main reservoir, $\delta^{15}N_{NO_3}^{-}$ and $\delta^{18}O_{NO_3}^{-}$ values increase (Figure 4.2a). We found a positive correlation between $\delta^{15}N_{NO_3}^{-}$ and $\delta^{18}O_{NO_3}^{-}$ isotopes ($r=0.5$). In the case of shallow groundwater from wells, isotopic values are close to zero (Table 4.1), reflecting nitrate from reduced N fertilizers and from soil organic nitrogen.
The relationship between $\text{NO}_3^-$/$\text{NO}_2^-$ and $\delta^{15}\text{N}_{\text{NO}_3^-}$ values followed the Rayleigh curve (Mariotti et al., 1981; Mariotti, 1984) with a negative enrichment factor ($\varepsilon = -2.9\%$) expressed by the regression analysis shown in Figure 4.3. According to the Rayleigh equation, denitrification contributions ranged from 7.8 to 49%, both maximum and minimum values were recorded during the wet period (March, 2009), with a total average of 16 ± 5%. (Table 4.1, Figure 4.2b). When we observed the relation with nitrate and $\delta^{15}\text{N}_{\text{NO}_3^-}$ in groundwater wells we found a lower negative enrichment factor (-2.3 %) compared to the observed in streams (Table 4.1).

### 4.3.2 Nitrate and ammonium concentrations from reactive transport modelling

The simulation of nitrate and ammonium transport and reactions in the main stream using the model showed a reasonable comparison with measured data (Figure 4.4a, 4.4b and 4.4c) through the length of the main stream (Chaminé stream). Parameters values were manually calibrated in the biogeochemical model with measured data for 2009, and subsequently also used for 2008 and 2003, except for the upstream boundary condition of ammonium which was varied for each period. Best-fit parameter values were 2.5 $\text{d}^{-1}$ for $r1$, and 25 $\text{d}^{-1}$ for $r2$. The trends showed that $\text{NO}_3^-$ and $\text{NH}_4^+$ decreased with distance from upstream ($\sim 17 \text{ mg N L}^{-1}$) increased in the second and third sampling point ($\sim 10 \text{ mg L}^{-1}$) and decreased to the reservoir equivalent to 0.5 mg L$^{-1}$. This trend had been similar during every run (Figure 4.4). It can be seen that the model reproduces the data reasonably well, especially for the validation year 2003. In 2008, the model predicts too low concentrations of nitrate.

### 4.4 Discussion

Nitrate was, most of the time, the major ion compared to $\text{NH}_4^+$ and $\text{NO}_2^-$ in the shallow streams, in spite of the fact that its concentration decreased downstream, until it almost disappeared, towards the receiving waters (Roxox reservoir). Nitrate consumption is clearly occurring in the streams and in waters reaching the reservoir, as can be seen in Figure 4.2a and Figure 4.4. Incoming nitrate concentration in the upstream dropped down to <0.1 mg N L$^{-1}$ while an increase in the isotopic signal $\delta^{15}\text{N}_{\text{NO}_3^-}$ and $\delta^{18}\text{O}_{\text{NO}_3^-}$ was observed downstream and during each studied period.
Records at the first sampling point showed very high ammonium values due to a considerably high ammonium input coming from wastewaters from the treatment plant outfall; located at ~ 500m upstream from our sampling point. Systematically, ammonium levels sharply decreased downstream, showing that it was rapidly consumed and converted into nitrate. The rise of nitrate concentrations accompanied by the drop in ammonium suggests nitrification is the most important process that occurs upstream. Note that this conversion of ammonium to nitrate was not reflected in the oxygen concentrations that strongly increased between the first two sampling points; presumably oxygen increases downstream due to re-aeration (Figure 4.1). The nitrate produced through nitrification was later denitrified to nitrogen gas, which lead to a gradual increase in the $\delta^{15}$N$_{\text{NO}_3^-}$ isotope abundance in the nitrate pool (Böttcher et al., 1990; Vitousek et al. 1997; Aravena and Robertson, 1998; Chen et al., 2009).
Table 4.1. Statistics of measured values for the streams and wells

<table>
<thead>
<tr>
<th></th>
<th>October 2008</th>
<th>March 2009</th>
<th>September 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO₃</td>
<td>NH₄</td>
<td>δ¹⁵N</td>
</tr>
<tr>
<td>Max</td>
<td>16.5</td>
<td>10.7</td>
<td>11</td>
</tr>
<tr>
<td>Min</td>
<td>1.27</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Av</td>
<td>7.24</td>
<td>1.2</td>
<td>7.5</td>
</tr>
<tr>
<td>SD</td>
<td>5.45</td>
<td>3.6</td>
<td>2.9</td>
</tr>
<tr>
<td>N</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>October 2008</th>
<th>March 2009</th>
<th>September 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO₃</td>
<td>NH₄</td>
<td>δ¹⁵N</td>
</tr>
<tr>
<td>Max</td>
<td>20.2</td>
<td>4.5</td>
<td>n.a</td>
</tr>
<tr>
<td>Min</td>
<td>1.7</td>
<td>0</td>
<td>n.a</td>
</tr>
<tr>
<td>Av</td>
<td>10.3</td>
<td>0.8</td>
<td>n.a</td>
</tr>
<tr>
<td>SD</td>
<td>8.18</td>
<td>1.8</td>
<td>n.a</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

n.a.: not available
The modelling part was included to assess the consistency of the observed data and to evaluate the impact of a long-stream transport compared to the biogeochemical transformations. To this end, we compared the values of nitrate and ammonium against the results from the reactive transport applied under steady state water flow conditions, and for the three sampling campaigns in the years 2003, 2008 and 2009. Assuming that the rise of nitrate and concomitant
decline of ammonium was due to nitrification was sufficient to reproduce the observed concentration gradients in the first section of the stream. The intense nitrification then led to the highest nitrate levels at the next site (A2). To reproduce the nitrate decrease downstream, a simple first-order consumption of nitrate was used. Processes that might remove nitrate from the stream are sediment and water column denitrification and assimilation by primary producers. Whereas the model does not distinguish between the actual process responsible for nitrate removal, both the enrichment factor and the degree of denitrification using Eq. (4.2-4.4, Table 4.1) as estimated using the equations in (Mariotti 1981; Mariotti 1984a; Kendal and McDonnell; 1998 Chen et al., 2009). Figure 4.2b confirmed that denitrification was occurring in the streams. However, denitrification was shown to be spatially variable, with low degrees of denitrification (< 10 %) recorded at some stream sample points (Figure 4.2b), suggesting that denitrification at these sites is less important than assimilation processes.

The streams in Roxo catchment are characterized by low flows and low water depths, mostly with high nitrate levels and it is well known these characteristics allow major ion exchange with the water-sediment interface (Erikson 2001; Sebilo et al., 2003). Eriksson (2001) through several microcosm experiments, using stream water and sediments from stream pools, identified that stagnating water and low water flow velocity conditions trigger higher denitrification rates. Sebilo et al (2003) hypothesised the diffusion of nitrate from the shallow stream water across the sediment-water interface is the limiting step in denitrification, producing a low isotopic signal in the stream water.

Pore water nitrate measurements from core sediments were obtained from three pools with rhizon samplers (Figure 4.6). In the first pool (P1), located near A3 sampling point, high nitrate values were found near the sediment-water interface and nitrate was strongly decreasing with depth, suggesting significant nitrate consumption in the sediment.
Stream denitrification using isotopic analysis and 1D reactive transport modelling

Figure 4.6. Average pore water measurements in three pools along the main stream Chaminé River. P1, P2 and P3 are the pore water-sediment cores in each sampling points.

In the second and third pool (P2 and P3) lower nitrate values (0.90 ± 0.37 mg L⁻¹) were found for the first centimetres, and the denitrification in the sediment was probably limited by nitrate there. At these sites, the diffusion through the water sediment interface to the pore water is apparently an important process influencing the kinetics of the denitrification occurring in the stream sediments. Besides, apparent enrichment factor (ε = -2.9 ‰) recorded in this study is considered rather low compared with experimental studies. However, it is not uncommon to find such low values in the environment (Lund et al., 2000; Sebilo et al., 2003; Fukuhara et al., 2007; Søvik and Mørkved, 2008). The difference in the enrichment value is commonly attributed to different substrate, temperature, denitrification rates, organic matter and biodiversity.

The model was used to estimate the nitrate production and consumption in the stream for the studied period (Table 4.2). Most of the nitrate in the stream was produced in situ; nitrification was 6 (2009) to 15 times (2003) higher than import from upstream. Nearly all the nitrate imported upstream or produced in the upstream part was consumed downstream (> 98%), such that only a very small fraction was exported downstream. In 2008, the model even predicted a very small import of nitrate from downstream.
Table 4.2. Average predicted nitrate stream export of nitrate from the Chaminé river to the Roxo reservoir. Units are in kg day⁻¹.

<table>
<thead>
<tr>
<th>Year</th>
<th>Production a</th>
<th>Consumption b</th>
<th>Stream Import c</th>
<th>Stream Export d</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>449.9</td>
<td>-469.6</td>
<td>28.7</td>
<td>9.03</td>
</tr>
<tr>
<td>2008</td>
<td>248.3</td>
<td>-248.5</td>
<td>27.5</td>
<td>- 8.69</td>
</tr>
<tr>
<td>2009</td>
<td>395.9</td>
<td>-453.2</td>
<td>62.7</td>
<td>5.46</td>
</tr>
</tbody>
</table>

a. Nitrate production by nitrification  
b. Nitrate consumption by denitrification  
c and d. Nitrate input and output at the upstream and downstream system boundaries.

The removal of nitrogen in the stream, strongly affected the N:P ratio in the Roxo reservoir. The very low N: P ratio were found in September-October 2008, March-April 2009 and August-October 2009 (0.3 mg N L⁻¹ : 0.04mg P L⁻¹), (0.3 mg N L⁻¹: 0.03 mg P L⁻¹) and ( 0.2 mg N L⁻¹: 0.09 mg P L⁻¹) respectively, indicated that N rather than P was the limiting nutrient then. This was supported by the observation that cyanobacteria comprised the major primary producers (Galvão et al., 2008; Valério et al., 2008; Valério et al., 2009). Also, it is likely that algae and denitrifying bacteria compete for this nutrient in the reservoir, such that the low nitrate concentration is maintained both by primary production and denitrification.

Figure 4.7 shows the isotopic composition of nitrate from different sources (Kendall and McDonnell, 1998) and superimposed on that the values as measured in the streams. The change in nitrate isotopic composition due to denitrification in the stream is added as a denitrification trend line (Figure 4.6 and Table 4.1). Nitrate from ammonium fertilizers and soil nitrate appeared to be the major sources to the streams in the upstream part. The denitrification then brings the isotopic composition in the area that is typical for manure septic systems and animal waste.
Figure 4.7. Ranges of nitrogen and oxygen isotopic composition of nitrate sources. The domain of soil nitrate is shaded (dark) and manure sources and ammonium fertilizers (lightest). The large line represents a denitrification vector; as denitrification progresses the nitrogen and oxygen isotopic values of the remaining nitrate progressively increase in the direction of the vector (Kendall and McDonnell, 1998).

Nevertheless, given the almost null precipitation, and the low stream flows, it is very unlikely that the sewage and manure contribute significantly during the summer.
Figure 4.8. Identification of hotspots of denitrification through the calculation of the degree of denitrification (Chen et al., 2009).

4.5 Conclusion

Both the standard chemical analysis of reactive nitrogen and the stable nitrate isotope data indicated that most of nitrogen inputs in the Roxo main stream waters are removed from the system and shallow waters by biogeochemical processes. The combination of the reactive transport model with biogeochemical data and the isotope signatures of the main compound (nitrate), permitted to identify sources, spatial location and rates of the nitrogen transformation and removal processes in surface water systems of the catchment. The combination of hydrochemical and stable isotope data analysis and interpretation with a reactive transport model permits to deepen our knowledge in the reactive nitrogen transport in aquatic systems, influenced by agriculture and other human activities.
Stream denitrification using isotopic analysis and 1D reactive transport modelling
Chapter 5

Abstract

Landsat TM data were used to map chlorophyll-a (Chla) concentrations in the Roxo reservoir, declared a vulnerable area by the European Nitrate Directive (91/676/EEC). A spectral mixing model was established based on endmembers generated from measurement data and spectral radiance earth observation data. The results showed a weak relationship between Landsat image radiances and our chlorophyll measurements ($R^2 = 0.30$). However, by taking into account suspended sediments, the disturbing spectral influence from suspended sediment was reduced by using the 2-component unmixing model, which predicted the SSC and chlorophyll in the water fairly well ($R^2 = 0.65$, $p = 0.016$ and $R^2 = 0.75$, $p = 0.005$). Furthermore, to indirectly support the predicted chlorophyll concentrations by the model, we plotted historical data of chlorophyll and sediment (2000 - 2009), and we found a very strong relationship between both parameters ($R^2 = 0.71$, $p = 4.19E-12$) with time. In parallel, we also compared the temporal chlorophyll-a concentration against nitrogen and phosphorus concentrations; we found that nitrogen acted as a limiting nutrient in the reservoir and phosphorus was present in excess. Therefore, the increasing knowledge of the spatial chlorophyll-a distribution, in the Roxo reservoir, may help to improve the description of the main features of the nutrient cycle in the reservoir.

This chapter is based on:
5.1 Introduction

Over-enrichment by nutrients of freshwater ecosystems in South Portugal in the last 25 years has resulted in eutrophication in various artificial water bodies used for human consumption. With the implementation of the EU Water Framework Directive there is a need for better knowledge on water quality and processes in the water reservoirs in Portugal by 2015. One of the freshwater bodies with eutrophication problems is the Roxo reservoir, which is an important drinking water reservoir for Beja city, and an irrigation source for adjacent catchments. One of the most objectionable symptoms of eutrophication in this reservoir is the noticeable shift in phytoplankton composition towards blooms of cyanobacteria, often nitrogen (N) fixing cyanobacteria, mainly in summer and autumn, resulting in high turbidity and a health risk for drinking water supply due to their toxins (Galvão et al., 2008, Schindler et al., 2008, Valério et al., 2009).

Increased nutrient availability in a water reservoir usually leads to a rise in chlorophyll concentrations in the water due to increased phytoplankton biomass. However, algal biomass can rapidly deplete nutrients to levels which would be difficult to sample and analyse directly. It is well known that chlorophyll-a (Chla) concentration is commonly used as an indicator of algal blooms (Carlson 1977, Morel and Gordon, 1980, Klemas 2012). Chla is a pigment with spectral characteristics that may be measured by optical instruments after extraction and also in vivo, and can be detected by remote sensing (Zhang et al., 2009). Nevertheless, effective remote retrieval of Chla is a major challenge in eutrophic turbid waters; the mixing of total chlorophyll and suspended sediment in satellite images can result in the wrong identification or overestimation of chlorophyll.

Recent advances in remote sensing methods that involve hyperspectral imagery, to quantify nutrients over-enrichment and chlorophyll of freshwater ecosystems, have improved knowledge and offered progress in deriving broad scale inputs to water quality and biogeochemical models (Kulkarni et al., 2008; Randolph et al., 2008). Yet, these approaches are still very expensive. However, several remote sensing studies have shown that Chla concentrations can be estimated as well from freely available multispectral Landsat imagery at levels of variation comparable with those of traditional ground-based monitoring (Torbick et al.,
A water pixel from a Landsat image contains information about its main optically active components (i.e., chlorophyll and suspended matter). These components are mixed together and the pixel radiance or reflectance observed by the sensors is a combination of radiance or reflectance of individual constituents, respectively. Therefore, spectral unmixing techniques should be used for separating these constituents.

The potential to combine notions from different disciplines, i.e., biogeochemical processes (nutrient excess or limitation) and remote sensing (i.e. Chlorophyll retrieval algorithms) to explain dynamics of nutrient enrichment in the Roxo reservoir, suggested us to apply an innovative approach based on a linear spectral mixing model by Shen and Verhoef (2010). They originally proposed this method to separate the interference of local atmospheric haze variations from sediment variations in the water. This approach is based on a linear spectral mixing model in which the measured spectrum of a mixed pixel is decomposed into end-members and their respective concentrations or abundances (Keshava and Mustrad, 2002).

This study aimed at demonstrating the applicability, in a turbid water reservoir, of a chlorophyll-a (Chla) retrieval algorithm based on the linear spectral mixing model discussed above. The method will be used to separate the chlorophyll and sediment variations in the Roxo reservoir with Landsat TM imagery, as a tool to identify the spatial chlorophyll distribution. Furthermore, we related historical Chla data with nutrient concentrations to identify nutrient availability in the water’s reservoir.

### 5.2 Materials and Methods

#### 5.2.1 Study area

The study was carried out in the Roxo Reservoir (37°55′48″ N and 8°6′9″ W), located in the Roxo catchment (352 km²), and the area covered by the reservoir is 13.78 km² (Figure 5.1). The construction of the reservoir took place in the period from 1963 to 1968. The catchment’s Roxo reservoir has a high economic importance, as the water is used for irrigation and public water supply purposes. The reservoir experiences eutrophic conditions, and during summer and autumn (from June until December) it is dominated mainly by blooms of cyanobacteria (Galvão et al., 2008, Valério et al., 2009).
5.2.2 Landsat TM imagery

Seven cloud-free Landsat TM images of the Roxo reservoir (13.78 km²) were downloaded from the U.S Geological Survey, Global Visualization Viewer site (http://glovis.usgs.gov/), from the 2002-2009 periods. ENVI software was used to process each Landsat image and to retrieve radiance values. Multispectral images were integrated for visual display, as true colour composites (bands 3, 2, 1) and false colour composites (bands 4, 3, 2). A linear spectral mixing model was developed using chlorophyll measurement data and the corresponding radiance values from Landsat TM imagery using bands 1, 2, 3 and 4. The algorithm was applied to the Landsat ETM+ image from September 6th, 2009, to extract chlorophyll and sediment values from the Roxo reservoir.

5.2.3 Surface water sampling

Historical data sets (from 2002 - 2008) from surface water samples of Chla, Nitrate, Phosphate, Total Phosphorus Dissolved Oxygen concentrations, and Secchi Disk Depth were used in this study. Information is available at the Sistema Nacional de Informação de Recursos Hídricos (http://snirh.pt). Additionally, surface water samples were also collected at ten sites in the reservoir. At each sampling site, water samples were collected and analysed for chlorophyll concentration, solutes, and suspended sediment and processed in the laboratory within a few hours after collection. All samples were filtered into glass-fiber filters (Whatman GF/F), extracted in 90% acetone, and left overnight at 4ºC in the dark. Chla concentration was determined fluorometrically (Holm-Hansen et al., 1965), following clarification of the extract by centrifugation for 3 minutes at 3000 rpm. The concentration of total suspended solids (TSS) was determined by filtering a known volume of water sample into GF/F filters and drying the filters for 24 h at 105 C.

5.2.4 Spectral mixing model

The aim of the spectral mixing model used is to decompose the measured spectrum into components related to chlorophyll and sediment, based on known endmember spectra for these components. These components have different spectral colours; therefore it is possible to apply a spectral unmixing to a given spectrum to decompose the signal into two components, chlorophyll and sediment. The selection of endmembers is an essential step and can be established in various ways, such as visual selection from the image by the user,
Spectral mixing model for mapping chlorophyll-a

from physical modelling results, or by using field measurements. Visual selection from images is subjective and not quantitative, since no *in situ* data are used.

![Sampling points in the Roxo reservoir](image)

Figure 5.1. Sampling points in the Roxo reservoir

In Shen and Verhoef (2010), a coupled radiative transfer model of the water-atmosphere system was used to generate the endmembers. This method is quantitative, but requires fitting of the model to the atmospheric circumstances of the moment. If, for a number of locations in the image the corresponding concentrations of chlorophyll and sediment are known from field measurements of the same day, the mixing model can be derived from these measurements and can also be used to generate artificial endmembers from the empirical model. This last method was applied in this particular study. In this case it was assumed that the atmosphere was clear and spatially homogeneous.
Suppose we have $m$ measurements of chlorophyll, sediment and the radiance measured by a satellite in a band $B$. We apply a linear model:

$$b = pc + qs + r$$  \hspace{1cm} (5.1)

where, $p$ = sensitivity of band $B$ to chlorophyll,
$q$ = sensitivity of band $B$ to sediment, 
$r$ = offset, 
$c$ = measured chlorophyll concentration, 
$s$ = measured sediment concentration (or turbidity), 
$b$ = radiance in band $B$

The offset $r$ is particularly needed to take account of atmospheric effects. The two sensitivities $p$ and $q$ express the sensitivities to chlorophyll and sediment variations, but also include all combined effects related to sensor calibration and atmospheric transmittance.

We organize all $m$ measurements into vectors of length $m$:

$$\begin{bmatrix} b_1 \\ \vdots \\ b_m \end{bmatrix} = p \begin{bmatrix} c_1 \\ \vdots \\ c_m \end{bmatrix} + q \begin{bmatrix} s_1 \\ \vdots \\ s_m \end{bmatrix} + r \begin{bmatrix} 1 \\ \vdots \\ 1 \end{bmatrix}$$  \hspace{1cm} (5.2)

Or, written as vectors in bold:

$$\mathbf{b} = \mathbf{p} \mathbf{c} + \mathbf{q} \mathbf{s} + \mathbf{r} \mathbf{1}$$  \hspace{1cm} (5.3)

Pre-multiplication by the transposed vectors of $\mathbf{c}$, $\mathbf{s}$, and $\mathbf{1}$ gives

$$\begin{eqnarray*}
\mathbf{c}^\top \mathbf{b} &=& p \mathbf{c}^\top \mathbf{c} + q \mathbf{c}^\top \mathbf{s} + r \mathbf{c}^\top \mathbf{1} \\
\mathbf{s}^\top \mathbf{b} &=& p \mathbf{s}^\top \mathbf{c} + q \mathbf{s}^\top \mathbf{s} + r \mathbf{s}^\top \mathbf{1} \\
\mathbf{1}^\top \mathbf{b} &=& p \mathbf{1}^\top \mathbf{c} + q \mathbf{1}^\top \mathbf{s} + r \mathbf{1}^\top \mathbf{1}
\end{eqnarray*}$$  \hspace{1cm} (5.4)

From this we can solve the sensitivities $p$ and $q$ and the offset $r$ for this particular band:

$$\begin{bmatrix} p \\ q \\ r \end{bmatrix} = \begin{bmatrix} \mathbf{c}^\top & \mathbf{c}^\top \mathbf{s} & \mathbf{c}^\top \mathbf{1} \\ \mathbf{s}^\top \mathbf{c} & \mathbf{s}^\top \mathbf{s} & \mathbf{s}^\top \mathbf{1} \\ \mathbf{1}^\top \mathbf{c} & \mathbf{1}^\top \mathbf{s} & \mathbf{1}^\top \mathbf{1} \end{bmatrix}^{-1} \begin{bmatrix} \mathbf{c}^\top \mathbf{b} \\ \mathbf{s}^\top \mathbf{b} \\ \mathbf{1}^\top \mathbf{b} \end{bmatrix}$$  \hspace{1cm} (5.5)

Now, for $n$ spectral bands we can apply the same model and write:
\[
\begin{bmatrix}
 b_1 \\
 \\
 b_n
\end{bmatrix}
= \begin{bmatrix}
 r_1 \\
 \\
 r_n
\end{bmatrix}
+ C \begin{bmatrix}
 p_1 \\
 \\
 p_n
\end{bmatrix}
+ S \begin{bmatrix}
 q_1 \\
 \\
 q_n
\end{bmatrix}
\]

(5.6)

where all sensitivities and offsets are now known for all bands, and where \( C \) and \( S \) are the unknown concentrations of chlorophyll and sediment of a given pixel with radiances \( b_1, \ldots, b_n \). The actual endmembers used are the sensitivity vectors \( p \) and \( q \), and the mixing model is written as a vector equation which includes information for all spectral bands:

\[
b = r + C p + S q
\]

(5.7)

or

\[
b - r = C p + S q
\]

(5.8)

and

\[
p^T (b - r) = C p^T p + S p^T q
\]

\[
q^T (b - r) = C q^T p + S q^T q
\]

(5.9)

from which we can obtain the final solution

\[
\begin{bmatrix}
 C \\
 S
\end{bmatrix}
= \begin{bmatrix}
 p^T p & p^T q \\
 q^T p & q^T q
\end{bmatrix}^{-1}
\begin{bmatrix}
 p^T (b - r) \\
 q^T (b - r)
\end{bmatrix}
\]

(5.10)

Note, that when this mapping equation is applied to a Landsat image, all vectors except \( b \) (which contains the band radiances of a pixel) are constants, so the predicted concentrations \( C \) and \( S \) are simply linear combinations of the Landsat bands.
5.2.5 Statistical analysis

A cross-validation method (leave-one-out) was used to predict the property value for every sample from the data set, which in turn is predicted from the regression equation calculated from the data for all other samples (Shao, 1993). For evaluation of these predicted values we used a scalar measure of fit, the standard root-mean-square error (RMSE). The RMSE is given by:

$$RMSE = \sqrt{\frac{1}{n} \sum_{i=1}^{n} (x_i - x_i^*)^2}$$  \hspace{1cm} (5.11)

where \( n \) is the number of points, \( x_i \) is the measured value and \( x_i^* \) is the predicted value for that point.

5.3 Results

5.3.1 Chlorophyll-a and physical and chemical parameters in the Roxo reservoir

The range of Chl-a concentration in the Roxo reservoir (2002 - 2007) measured values was from 1.9 to 191 mg m\(^{-3}\), and the average for this period was 29.8 ± 40.8 mg m\(^{-3}\). Chl-a concentration was very high during summer and winter periods and showed higher peaks (> 100 mg m\(^{-3}\)) in June and November. The lowest Chla concentrations occurred in January and February 2002 with values reaching 1.9 mg m\(^{-3}\) (Figure 5.3). Transparency readings ranged from 0.3 to 3.2 m (2002 - 2009) and these were non-linearly correlated with Chl-a (\( R^2 = 0.24, p = 0.016, n = 80 \)).
5.3.2 RGB imagery

We illustrated eight multispectral images of Landsat TM from 2002 until 2009 as true colour composites and the same number as false colour composites (Figure 5.4a and 5.4b). The RGB visible bands combination (3, 2, 1) simulates a natural colour image (Figure 5.4a). These Landsat images from the Roxo reservoir show the changes that occurred in the lake level between 2002 and 2009. The early image in the spring of 2002 still shows a healthy supply of water, but late in 2009, water levels had dropped drastically. The second RGB combination (4, 3, 2) corresponding to the false colour combination combines near-infrared band 4 and the visible bands 3 and 2. The use of Band 4 results in a better definition of the shorelines than using bands 3, 2, 1. In these images, the Roxo reservoir is clearly visible and sediment loads are substantially more evident. In general, the light blue colour indicates more suspended sediment material and dark blue colours indicate a higher transparency of the water.
Figure 5.3. Nutrients and chlorophyll-a time series data in the Roxo reservoir.
5.3.3 Separation of suspended sediment and chlorophyll variations in Landsat corrected radiance.

Maps of sediment/chlorophyll variations in the Roxo reservoir were obtained as the result of the spectral unmixing algorithm applied to Landsat corrected radiance data from the September 6, 2009 image, and are shown in Figure 5.4a and Figure 5.4b. The maps indicate that sediment has a larger spatial variation than chlorophyll, but otherwise both concentrations appear to be correlated quite strongly. We could ensure that the model has estimated moderately well the sediment concentrations in the water reservoir ($R^2 = 0.75$, $p = 0.005$, Figure 5.6b). Historical data sets of chlorophyll and sediment confirm the strong relation between them ($R^2 = 0.71$, $p = 4.19E-12$) and help to indirectly validate our predictions (Figure 5.7).

Figure 5.4. Landsat ETM+ RGB Imagery on eight dates. a) True colour combination (321, left), b) False colour combination (432, right).

100
Figure 5.5. Maps of a) SSC (mg L\(^{-1}\)) and b) Chlorophyll (\(\mu\)g L\(^{-1}\))

The cross-validation method (leave-one-out) predicted the property value for every sample in the data set and we obtained root mean square errors (RMSE) value for chlorophyll and sediment of 0.23 and 5.84, respectively.
Figure 5.6. Scatter plots for September 6th, 2009, estimated versus measured, values of chlorophyll (left) and turbidity (right).

Figure 5.7. Historical relation (2002-2009) between chlorophyll and suspended sediment concentrations in the surface water of Roxo reservoir.

5.4 Discussion

5.4.1 Chlorophyll turbidity and model.

The purpose of this study was to demonstrate the applicability of a chlorophyll-a (Chla) retrieval algorithm based on a linear spectral mixing model as a tool to identify the spatial chlorophyll distribution (as an indicator of algal biomass). In
doing so, we employed an approach suggested by Shen and Verhoef, 2010, coupled with the application of a spectral unmixing algorithm to separate chlorophyll concentrations from sediment concentrations in turbid and eutrophic waters from the Roxo reservoir.

Because the studied water reservoir is a case 2 type of water, it may contain enough suspended sediment in known concentrations to interfere with chlorophyll-a mapping. This is the cause that a good relationship between the radiances of Band 3 of Landsat images (Figure 5.9) and our chlorophyll measurements ($R^2 = 0.30$) was not found. However, the disturbing spectral influence from suspended sediment was reduced by using the unmixing model which predicted well the SSC and chlorophyll in the water ($R^2 = 0.65$, $p = 0.016$ and $R^2 = 0.75$, $p = 0.005$; Figure 5.6). Moreover, to indirectly support our model, we plotted the historical data sets of chlorophyll and sediment (2000 - 2009) and found a very strong relation between both parameters ($R^2 = 0.75$, $p = 4.19E-12$, $n = 65$; Figure 5.7).

Chlorophyll-a values extracted from Landsat images can be the result of other constituents which absorb light. Hence, Secchi disk depth has helped to measure the light attenuation (transparency) and also estimate the potential relation with Chlorophyll-a. For instance, if phytoplankton would be the only factor determining the photic zone depth, then the relation between Chla and the Secchi disk depth (transparency) would be good. However, we found during the study only a weak relationship between both parameters (Figure 5.8a-b, $R^2 = 0.24$, $p = 0.04$, $n = 65$; $R^2 = 0.30$, $p = 0.04$, $n = 8$) which indicates that Chla is not the only factor absorbing light.

The chlorophyll spatial distribution in the reservoir, according to our estimations, showed high values in the arms of the reservoir and lower values in the middle of it. Similar patterns were observed for the suspended sediment concentration (Figure 5.5). The concentration pattern found corresponds also to those found and described by Folgôa-Batista et al. (2003) for the reservoir.
Spectral mixing model for mapping chlorophyll-a

Figure 5.8. Historical relation (2002-2009) between chlorophyll and suspended sediment concentrations in the surface water of Roxo reservoir.

Figure 5.9. Landsat multispectral data in bands 1 - 4 for eight sampling points. Radiances after minimum value subtraction.
5.4.2 Nutrient turbidity and limitation

Since it is known that high nutrient (nitrogen or phosphorus) concentrations stimulate excessive algal growth (> chlorophyll), the question arises whether nutrient concentrations in the reservoir could be inferred from or be related to the chlorophyll levels present in it. High levels of Chla recorded in the ground data, plus the spectral mixing model predictions, supported by historical data followed by ground nutrients measurements, showed low nitrogen values, in case of nitrate particularly close to zero and higher total phosphorus and phosphate concentrations (Figure 5.4a-b, 5.10). The latter, total phosphorous, is usually associated with turbidity due to the adsorption of phosphorus by clay particles (Grayson et al., 1996, Spackman, 2008), which explains why the values recorded in this study were higher in the more turbid waters of the reservoir (Figure 5.9). Several studies have identified intense cyanobacterial blooms in the Roxo reservoir which increased during summer and lasted until autumn (Galvão et al., 2008; Valério et al., 2008; Valério et al., 2009). Schindler et al. (2008) have indicated that reducing nitrogen levels in many lakes could cause blooms of nitrogen-fixing cyanobacteria.

Since Redfield’s observations showed that phytoplankton contained a molecular C:N:P ratio of 106:16:1, the use of these elemental ratios has become essential to estimate which nutrient limits algae growth, and has widely been used in freshwater studies. The Redfield ratio has often been used to establish whether nutrients supply is sufficient in the water (Schindler et al., 2008) and therefore, the ratio of Nitrogen: Phosphorus (N: P) has consequently been accepted as a general indicator for algal balanced growth.
If conditions of N: P ratio reduction are present in the water, it is potentially observable that cyanobacteria has begun to dominate the phytoplankton population at least during summer periods; conditions that have been recorded in the Roxo reservoir. (Galvão et al., 2008; Valério et al., 2008; Valério et al., 2009).

Historical data and our measurements showed that the N:P ratio was lower during the summer and autumn period (Figure 5.3). With these low N:P ratios, one would expect blooms of cyanobacteria beginning to occur in the Roxo reservoir. It is worth noting that relative abundances of cyanobacteria are close to 60% during summer and autumn (Valério et al., 2009), which means that these blooms are present during half of the year. This is very long compared to the typical patterns of algae succession in temperate lakes (Smith, 1983; Rocha et al., 2002; Valério et al., 2009).

Schindler (1974) and Schindler (1977) found that in many lakes where P was added, nitrogen limitation on the other hand was extreme. In both studies he concluded that nitrogen limitation was a symptom of over-fertilization with phosphorus. In our study we have shown that nitrate could reach low values in
the Roxo reservoir for the whole range of Chlorophyll-a (2 - 190 mg m\(^{-3}\)) and high historical Phosphorus values, which presents a strong relationship with suspended sediment concentration (Figure 5.10).

Therefore, the application of a chlorophyll-a (Chla) retrieval algorithm based on the linear spectral mixing model proved to be useful to separate chlorophyll and sediment variations in turbid water reservoirs. Thus, chlorophyll values obtained can be related to nutrient concentrations to identify the availability of nutrients and help to determine which management strategies are more likely to generate positive effects to control the excessive algal growth and improve the associated negative impacts to the ecosystem.

### 5.5 Conclusion

In this study, a spectral mixing model was implemented for turbid waters using Landsat imagery to separate chlorophyll from sediment effects in a drinking water reservoir in Portugal. Our results showed that the spectral mixing model gave good results in the model’s precision. Ground data and radiance values from the Landsat imagery were used to estimate chlorophyll-a concentrations with a spectral unmixing model. We were able to find in the historical data a strong correlation between chlorophyll and sediment, and this was confirmed by our model results. The results showed that configuring a spectral mixing model is feasible with a limited number of ground measurements, and can be used as a practical application of remote sensing for water quality monitoring.

With regard to historical data of the chlorophyll-a correlation with the nutrient ratio (N:P), our findings suggest that high chlorophyll-a values detected in the surface waters are followed by low nitrogen concentrations, particularly for nitrate, with concentrations close to zero. The fact that low nitrate values’ correlated with high Chlorophyll-a levels (2-190 mg m\(^3\)) suggests that nitrate during summer periods may act as the limiting factor in the reservoir, whereas the phosphate can be in excess and associated with suspended sediments. Our method then can help to assess the spatial distribution of Chlorophyll-a occurring in surface waters of the Roxo reservoir. Furthermore, it proved to be a valuable tool to increase knowledge towards the primary production and the spatial nutrient cycling in the reservoir.
Spectral mixing model for mapping chlorophyll-a
Chapter 6

Synthesis
6.1 Introduction

Nitrogen levels in streamflow are important indicators of environmental catchment conditions (Arheimer and Brandt, 1998; Mulholland et al., 2005; Piatek et al., 2009). A multitude of human activities, such as agricultural practices or urban residual waste water effluent releases, may produce an excess of nitrogen supply in a catchment, and can lead to increased nitrogen losses, especially in the form of nitrate-nitrogen (nitrate), thus disturbing and impacting the water quality of ecosystems (Ventura et al., 2008). Concern about nitrate impacts on freshwater bodies from activities such as agriculture dates back more than 40 years ago, when the US (Clean Water Act, 1972) but also the Commission of the European Community (CEC) became interested in maximizing the fertilizer potential of animal slurry applied to agricultural areas (Sluijsmans, 1978). Nowadays, it remains a concern in the European Water Framework Directive, whereby several agriculture-dominated regions across Europe have been classified in the European Nitrate Directive 91/676/CEE, as areas vulnerable to nitrate contamination from agricultural sources.

This thesis deals with the characterization of reactive nitrogen dynamics associated with biogeochemical and eco-hydrological processes in streams and a receiving water reservoir of an agricultural catchment. Hydro-chemical analysis including multi-isotope analysis ($\delta^{15}$N, $\delta^{18}$O and $\delta^{2}$H), eco-hydrological and geochemical modelling, statistical methods and satellite imagery were applied and integrated in a hydro-geochemical assessment of the fate of nitrate in stream water, sediment pore water, shallow groundwater and the receiving water reservoir at the catchment outlet. The use of different methodological approaches led to increased understanding of the N dynamics in small streams and reservoirs in agricultural areas.

The research area and study catchment is located in the Alentejo region in Southern Portugal (Figure 1.1 e.g. in Chapter 1). The Roxo streams and catchment are tributaries and sub-basin of the Sado river basin and drains a large part of the important agricultural Alentejo region of Southern Portugal. The research catchment presents the upper part of the Roxo catchment (352 km$^2$) near Beja city, and drains into a reservoir or “barragem do Roxo”, an impoundment which is the main source of the domestic water supply for Beja city, as well as the water supply for the local mining industry and important
irrigation areas (ABROXO, 2009). The reservoir has, however, been under considerable water stress for several years owing to the combination of inter-annual weather variability that affects natural rainfall supply and reservoir inflow, increased water consumption, and contamination threats of varying origins (UNEP, 1997). There is serious and continuous concern among the local and regional authorities regarding the Roxo reservoir, related to both water quantity and quality. The catchment area lies also within the zone of influence (i.e. for inter basin water transfers) of the large Alqueva dam and reservoir on the neighboring Guadiana river. Several large agricultural areas in the region have been classified as a vulnerable zone since 2006 according to the European Nitrate Directive 91/676/CEE.

Several field studies and data from the upper Roxo catchment area have indicated high nitrate concentration, around 15 mg N L$^{-1}$ in small streams and shallow groundwater (Chisha, 2003; Gurung, 2005; Vithanage, 2009). Chisha (2003). Gurung (2005) suggested that the Roxo reservoir is a hypertrophic system, because maximum nitrate concentrations of 14 mg N L$^{-1}$ and high values for other eutrophication indicators such as phosphorus and chlorophyll-a were regularly observed in the reservoir. Vithanage (2009) recorded NO$_3^-$ levels ranging from 2 to 13 mg N L$^{-1}$ in streams located in the southern part of the catchment, which in fact significantly exceeds the nitrate levels (5.65 mg N L$^{-1}$) established by the European Water Framework Directive (2000/60/EC). However, it is known that only a small percentage of the net nitrogen pool in a catchment is generally exported to streams (Boyer et al., 2002), while the rest is retained or lost in the watershed system through denitrification or volatilization into the atmosphere before reaching the water body (Filoso et al., 2003).

Next to increasing our scientific understanding of nitrogen processes and dynamics in small streams and impoundments draining agricultural catchments, this thesis also aimed to contribute with scientific advice to the local and regional authorities and populations on issues of fertilizer use, agricultural nutrient management and related water contamination issues in this agriculturally important region of Portugal.
6.2 Conclusions

As a general research finding, this thesis revealed that nitrate nitrogen is highly reactive in the riparian stream network of agriculturally impacted catchments like the upper Roxo, with significant and fast transformations occurring, like production by nitrification and subsequent removal or consumption by denitrification, mainly near the streambed (sediment pore) interface, as well as in stagnating water pools along the stream network and the water reservoir. This study also acknowledges that the study of nitrogen dynamics and nitrification - denitrification and coupling to other biogeochemical processes such as nitrogen fixation by algae and anammox processes in streams is required. This inherently requires integration across the hydrology and biogeochemistry disciplines and research at different scales, from laboratory to field and catchment. In accordance with the overall finding, three main focal points about reactive N transport in small streams could be identified:

1. Knowledge of hydrological flow paths in a catchment and water flow contributions from rainfall, soil and groundwater, and the role of the sediment pore water interface are key to understand nitrogen processes such as nitrification - denitrification in stream networks of watersheds, and the estimation of main eco-hydrological processes might significantly help to identify N dynamics of small streams that are strongly influenced by land-based inputs.

2. It is rather essential to identify the different environmental factors governing N dynamics and budget in order to quantify and potentially manage N inputs, and affecting the water quality of agricultural catchments.

3. The use of innovative combinations and coupling of different data analysis approaches to study nitrogen budgets in streams and catchments: such as stable isotopes analysis, eco-hydrological and geochemical modelling and geo-statistical analysis, is advantageous and permits to infer and confirm results found by a single methodology.

In Chapter 2, an eco-hydrological catchment modelling approach using the SWAT model was applied to estimate nitrate nitrogen exports from the catchment’s land areas to the water reservoir. Own measurements and detailed (daily) historical chemical, climate and physical (2001–2008) datasets of
rainfall, surface waters, shallow groundwater and soils were used in the modelling. The results suggested that seasonal fluctuations and agricultural crops play an important role in the variation in nitrate loss via the streams towards the Roxo reservoir. Meanwhile, the catchment nitrate budget analysis indicated the largest inputs come from nitrogen fertilizers, but biological fixation and wet deposition by rainfall can also be important N sources and contribute to the catchment nitrogen budget. In areas where denitrification naturally reduces nitrate levels in soils and streams, the largest contribution to stream nitrate originates from leached soil nitrate, re-appearing in the groundwater baseflow. Contributions from shallow groundwater was very significant, compared to less than 2% from direct surface runoff during high rainfall events. A nitrogen fertilization reduction scenario was implemented in the modelling to evaluate remedial nitrate control policies which could be implemented as potential control strategies for stream restoration. The scenario was adjusted according to the European Nitrate and Water Framework Directives the requirements. Moreover, Chapter 2 tackled and estimated the different contributions of nitrate to the streams. The analysis and findings presented in this chapter, gave a general baseline of the catchment nitrate budget and the stream nitrate exports to the main water reservoir based on recent and historical chemical, climatic and bio geophysical data.

The understanding and knowledge of the origins of the nitrate sources and source water contributions to the streamflow are crucial in chemical contamination studies. In Chapter 3, the study tackled the origins of the nitrate sources and hydrological pathways in the catchment. An end member (un)mixing analysis approach was used, in combination with residual analysis to determine the proportional contributions from rainfall, sediment pore water and shallow groundwater to the streamflow and receiving water reservoir. A combination of chemical ions and the stable water isotope signatures were used as tracers to identify source contributions.

The use of a few seasonal campaigns (wet and dry season) in combination with a relatively limited number (~30 points) of spatially distributed sampling points was also evaluated as a practical method to carry out this source deduction analysis. This method has been only applied or is documented for small research catchments with continuous streamflow and chemical observations.
Different data scenarios were tested to distinguish proportional water and nitrate source contributions to streamflow. The results indicated that in the upper Roxo catchment, source contributions to annual streamflow corresponded to 50% from shallow groundwater, followed by sediment pore water (~40%) and rainfall (~10%) respectively. Non parametric statistical tests also revealed that shallow groundwater, is directly related to stream nitrate concentrations. The high sediment pore water contributions to the streamflow chemical signature indicated that the water-sediment interface in the streambed zone plays an important role in the nitrate exchanges and regulation in the streamflow. Nitrate concentrations in stream water was significantly lower than in the shallow groundwater, which can be mostly explained by nitrate consumption (e.g. denitrification) in the stream sediment, during transfer from shallow groundwater to the stream during baseflow periods. In Chapter 4 this aspect has been discussed more in detail.

Chapter 4 build on the assumption, validated in Chapter 3, that agriculturally impacted small streams and the stream sediment-water interface, are important sites for nitrogen removal by processes such as denitrification. In order to decipher further the fate of nitrogen in the major stream of the upper Roxo catchment and main streamflow contributor to the reservoir, an open source R-code 1-D reactive hydrological transport model was built, with biogeochemical data and isotopic signatures of nitrate $^{15}$N/$^{14}$N, which allow discrimination between soil nitrate, manure and waste water as the main sources. Small drainage lines and streams and by preference their water - sediment interfaces in agricultural catchments can be viewed as preferred sites for rapid nitrification - denitrification processes. The main seasonal stream in the catchment (i.e. the Pisoes - Chaminé – Outeiro drainage system) was identified as an effective nitrification and denitrification zone, where residual ammonium releases from the waste water treatment plant are rapidly transformed to nitrate which is subsequently removed from the system by denitrification processes. In-stream geomorphic structures such as small stagnating water pools in the stream network had a significant impact on the N loads acting as "hotspots" for biogeochemical processing and denitrification. Therefore, N-removal is relatively high in the upper part of the stream, taken into account a decrease of nitrate concentration towards downstream and the sediment pore water trends results. In the Roxo reservoir, very low nitrate values are observed, but
contrastingly together with historical and frequent recurrences of algal blooms, suggesting that high nitrogen loadings are not a necessary requirement for this (eutrophication) phenomenon to occur in reservoirs.

Based on the findings on nitrogen reactive transport in the streams and inflows into the reservoir which were exposed in the previous Chapters 2, 3, and 4, in Chapter 5 we focused our attention on the Roxo reservoir lake. A remote sensing data based approach was adopted to analyse potential relationships or links between nutrient levels and algal blooms, regularly occurring in the lake body. A spectral model was built to identify chlorophyll-a levels (as a proxy for algae concentration) and to relate these values with historical data on nutrient availability. The spectral mixing model was established based on endmembers generated from field data and spectral radiance data. The model incorporated information derived from Landsat imagery to separate chlorophyll from effects from sediment in the Roxo reservoir. Historical data supported the model results with a strong correlation between chlorophyll and sediment and building a strong model’s precision. It is also remarkable from this chapter that configuring a spectral mixing model with a limited number of ground measurements is feasible, and can be used as a practical application of remote sensing for water quality monitoring.

The findings about nutrient availability suggested that high chlorophyll-a values detected in the surface waters are followed by low nitrogen concentrations, particularly nitrate, with concentrations close to zero. This suggest that nitrate during autumn-summer periods may act as the limiting factor in the reservoir, whereas phosphate can be found in excess and associated to suspended sediments. The method proposed here can help to assess the heterogeneity of Chlorophyll-a levels occurring in surface waters of the Roxo reservoir. Furthermore, it proved to be a valuable tool to increase knowledge towards the primary production and the spatial nutrient cycling in the reservoir.

6.3 Limitations

This thesis provides insights into the biogeochemical transport of reactive nitrogen and removal in the stream network of an agricultural catchment. The methods developed and applied in this thesis permitted to better identify hotspots of denitrification, as well as identification of the nitrate source origins and the nitrogen export to the main receiving water body (reservoir) at the outlet
of the catchment. Therefore, these findings can help to improve water quality management and make it more effective through the use of a combination of hydrological analysis and biogeochemical models.

However, some limitations of the study must be mentioned. An issue which could not be fully addressed in this thesis is the high temporal variability of the studied phenomena and also present in the data and observation campaigns. Streams in catchments of this size (320 km²) and especially in these Mediterranean climates are affected by large temporal variations in weather, hydrological and biogeochemical conditions. This aspect was addressed in Chapter 2 using daily weather and rainfall streamflow records. However, the other analysis was based on seasonal or shorter sampling campaigns in the dry and wet seasons and typical (e.g. dry weather flow at the end of summer, etc.) conditions were assumed. We are fully aware that more continuous sampling in short time intervals could have improved the results. However, the study also aimed to verify whether more short and limited spatial sampling campaigns and datasets could permit to analyse nitrogen processes in streams of catchments.

6.4 Future research

This thesis investigated nitrate nitrogen dynamics, transport and export in the stream network of an agricultural catchment located in Southern Portugal. The research provides useful information on whether these agricultural catchment systems are sources or sinks for nutrients. Improving on the quantification of nitrogen processes and budgets is particularly important, also in the upper Roxo catchment due to the objectives of European Nitrate Directive, which prescribes a reduction and prevention of contamination in vulnerable areas, and from emission sources like urban residual waste water treatment plants or from agricultural activities respectively.

This study recommends further research in order to capture better the temporal variability, and could be addressed by analysing the streambed function in relation to stream N cycling. For example, evaluating how changes in stream hydrologic conditions (e.g., stream flow rates) alter streambed exchange rates and residence times that control denitrification potentials. Or, how do variations in stream and groundwater chemistry (e.g., seasonal changes in NO₃⁻, temperature amongst others) affect the redox conditions that ultimately control denitrification. For this it is necessary to keep in mind the analysis at different
spatial and temporal scales, as well as to combine different approaches from various scientific disciplines i.e. the biogeochemistry, hydrology and climate sciences.
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Summary

Reactive nitrogen (N) originating from agricultural catchments is a major source of N loadings to streams and groundwater in numerous regions in Europe and around the world (European Directive, 2000, Balestrini et al., 2007).

Concerns about environmental impacts of nitrate on freshwater systems and protection of water quality have led to the European Water Framework and Nitrate Directives (European Nitrate Directive, 1991; European Directive, 2000). The member states in Europe are obliged to implement these legal frameworks within a pre-set timeframe like 2015 for the EU-WFD.

The upper Roxo study catchment is located in the Alentejo region in Southern Portugal and presents a historically intensively cultivated agricultural area. The upper catchment is bounded downstream by a medium scale water reservoir, providing drinking and industrial water to local populations and irrigation water to large irrigation areas. The catchment and surroundings are classified as vulnerable zone by the Nitrate Directive, and also lie within the zone of influence of the large Alqueva dam on the Guadiana River. Important agricultural developments and irrigation area extensions are planned using inter basin transfers. This makes research on water quality and environmental protection of water resources relevant for this region.

Nitrate can constitute a large fraction of the total dissolved nitrogen in streams, but the potential of ammonium and nitrate respectively available for nitrification and denitrification and/or potentially contribute to eutrophication in streams and drinking water reservoirs is not well known and quantified, also in the case of the upper Roxo catchment. Transformations of reactive nitrogen along terrestrial water flow paths may alter its bioavailability. Therefore, hydrology and biogeochemical processes together are likely to play an important role in the transport and fate of reactive nitrogen in streams and receiving water bodies like reservoirs.

This research aims to improve our understanding of the processes related to the delivery of reactive nitrogen to surface waters and impoundments from point and non-point sources. An interdisciplinary approach using hydrochemistry
including stable isotopes, hydrological and water quality modelling and satellite imagery was used in a coupled hydrological and biogeochemical assessment. This thesis used biogeochemical and hydrological modelling approaches and in the analysis of reactive nitrogen dynamics in shallow streams, shallow groundwater and the receiving drinking water reservoir of an agricultural catchment in Portugal.

The research was driven by field measurements, chemical analysis including the stable isotopes of water and nitrate, eco-hydrological and geochemical modelling and satellite data analysis. In order to achieve the overall aim, the research was divided in four objectives: (1) to estimate the nitrate exports by streamflow of an agricultural catchment to a receiving drinking water reservoir using an eco-hydrological catchment model (2) to detect water and nitrate source contributions to stream flow using a multivariate analysis and an end-member mixing modelling or EMMA approach (3) to jointly analyse stable nitrate isotopes within a 1D reactive transport model (4) to build and use a spectral mixing model using Landsat satellite imagery to estimate eutrophication related water quality parameters in the Roxo reservoir and relate these to nutrient inflows.

The methods were developed for this catchment but they can be applied to other streams in agricultural catchments after appropriate testing, calibration and validation.

The results from both the eco-hydrological SWAT model and end-member mixing analysis (EMMA) showed that in the catchment the largest contribution to stream nitrate is originating from leached soil nitrate, re-appearing in the groundwater baseflow. Denitrification processes are subsequently occurring and reduce nitrate levels in soils and the shallow streams.

The SWAT eco-hydrological model has proven to be a useful tool for screening of nitrogen loadings in the upper Roxo catchment. It also permitted to evaluate fertilizer management (reduction) scenarios in order to meet European legal water quality standards and requirements. Biogeochemical process representations however need to be further refined and included in the model,
before it can be used as a full hydrological and biogeochemical prediction system.

As indicated and confirmed by the EMMA analysis, the high sediment pore water exchanges and contributions to the streamflow chemical signature indicated that the water-sediment interface in the streambed zone plays an important role in the nitrate exchanges and regulation in the streamflow.

The end-member mixing analysis is good and straightforward approaches to infer sources origins of water and chemicals in catchments. A limited but well-designed spatial and temporal sampling scheme and chemical dataset can be used in the method.

The most impacted small streams and the stream sediment-water interface near to the waste water treatment plant outfall in the Pioes sub-catchment near Beja represent important stream sites for nitrogen removal by processes such as nitrification and denitrification. In certain stream segments, and especially those with significant ammonium levels, we do not exclude annamox reaction mechanisms to control stream nitrogen, together with nitrification and denitrification. Small drainage lines and streams and by preference their water-sediment interfaces in agricultural catchments can be viewed as preferred and important sites for rapid nitrification - denitrification processes.

The analysis and interpretation of multiple isotope signatures ($\delta^{15}N$, $\delta^2H$, $\delta^{18}O$) of both water and nitrate was done in combination with open source reactive transport modelling. This approach offers good scope for analysis of transport and fate of nutrients in catchments.

The upstream areas of the streams were identified as effective nitrification and denitrification zones, where e.g. residual ammonium releases from the waste water treatment plant are rapidly transformed to nitrate which is subsequently removed from the system by denitrification processes. Also nitrate present in the shallow aquifer and re-appearing in the baseflow undergoes significant denitrification in the stream flow and sediment water interface. Due to these processes, stream nitrate decreases downstream, and it almost disappears when streamflow reaches the receiving Roxo reservoir. Hence, in contrast to
conventional thinking, in this case of the Roxo reservoir, nitrogen and nitrate may act during certain periods as the limiting nutrient factor in the reservoir, whereas phosphorus is usually found in excess and associated to suspended sediments.
**Samenvatting**


De bovenloop van het Roxo stroomgebied dat als studieobject is gekozen bevindt zich in de Alentejo regio in Zuid Portugal en vertegenwoordigt een gebied met intensieve landbouw. De bovenloop wordt benedenstrooms begrensd door een middelmatig groot waterreservoir, dat drinkwater en industriewater verschaf aan de plaatselijke bevolking en tevens irrigatiewater voor grote bevlodieerde landbouwgebieden. Het stroomgebied en de omgeving ervan zijn aangewezen als kwetsbare zone door de Nitraat Richtlijn, en deze liggen ook binnen de invloedsfeer van de grote Alqueva dam, gelegen op de benedenstroom op de Guadiana rivier. Plannen voor belangrijke landbouwkundige ontwikkelingen en uitbreidingen van het geïrrigeerde oppervlak en bevloeiing worden onderling tussen waterbekkens afgestemd. Dit maakt het onderzoek van de waterkwaliteit en de bescherming van waterbronnen zeer relevant voor dit gebied.

Van de totale hoeveelheid opgeloste stikstof in een stroom kan nitraat een groot deel uitmaken, maar de potentiële hoeveelheden van ammonium en nitraat beschikbaar voor nitrificatie, respectievelijk denitrificatie, en/of eutrofiëring in stromen en drinkwaterreservoirs, zijn niet goed bekend en gekwantificeerd, en dat is ook het geval voor de bovenloop van het Roxo stroomgebied. Omzettingen van reactieve stikstof langs waterlopen in het landschap kunnen de beschikbaarheid voor biologische processen beïnvloeden. Daarom spelen hydrologische en biogeochemische processen waarschijnlijk een belangrijke rol in het transport en het verbruik van reactieve stikstof in stromingen en ontvangende waterbekkens.
Dit onderzoek richt zich op het vergroten van ons begrip van de processen die betrekking hebben op de levering van reactieve stikstof aan oppervlaktewater en waterbekkens afkomstig van zowel puntbronnen als meer diffuse bronnen. In een gekoppeld hydrologisch - biogeochemisch onderzoek is een interdisciplinaire benadering toegepast waarbij gebruik is gemaakt van hydrochemische technieken, waaronder stabiele isotopen, hydrologische modellen, waterkwaliteitsmetingen en satellietbeelden. In deze dissertatie zijn bij de analyse van de dynamiek van reactieve stikstof in ondiepe stromen, ondiep grondwater en het ontvangende drinkwaterreservoir verschillende biogeochemische en hydrologische modelbenaderingen gebruikt.

Het onderzoek is vormgegeven door veldmetingen, chemische analyse met stabiele isotopen van water en nitraat, eco-hydrologische en geochemische modellen en de verwerking van satellietbeelden. Om de algemene onderzoeksdoelstelling te bereiken zijn er vier deeltaken gesteld: (1) het schatten van de nitraatuitvoer door waterafvoer in een landbouwgebied naar een drinkwaterreservoir, door gebruik te maken van een ecohydrologisch model voor het bekken, (2) het detecteren van bijdragen van water- en nitraatbronnen aan de rivierstroom via multivariate analyse en een EMMA benadering met endmembers, (3) gezamenlijke analyse van stabiele isotopen van nitraat binnen een 1D reactief transportmodel en (4) het afleiden en gebruiken van een spectraal mengingsmodel met Landsat satellietbeelden om waterkwaliteitsparameters te schatten die te maken hebben met eutrofiëring van het Roxo reservoir, en deze te relateren aan de instroom van nutriënten.

De methoden zijn ontwikkeld voor dit stroomgebied, maar ze kunnen, na een proces van testen, kalibratie en validatie, ook worden toegepast op andere stromen in landbouwgebieden.

De resultaten van zowel het ecohydrologisch SWAT model als de EMMA ("end-member mixing analysis") resultaten tonen aan dat in het bekken de grootste bijdrage aan de stroom van nitraat komt van uitspoeling van bodemnitraat, dat daarna terugkomt in de grondwater uitstroom in het drainage netwerk. Vervolgens treedt er denitrificatie op, waarbij de nitraatniveaus in de bodem en in ondiepe stromen worden verlaagd.
Het SWAT ecohydrologisch model heeft zich bewezen als een bruikbaar gereedschap voor het bepalen van de nitraatbelasting in de bovenloop van het Roxo bekken. Het maakte ook mogelijk scenario’s voor het beheer (bijv. reductie) van meststoffen te evalueren om te kunnen voldoen aan Europese wettelijke waterkwaliteitseisen en standaarden. Representaties van biogeochemische processen moeten echter nog verder worden verfijnd en ingebracht in het model, voordat dit kan worden gebruikt als een volledig hydrologisch en biogeochemisch systeem dat geschikt is voor het maken van voorspellingen.

Zoals aangegeven en bevestigd door de EMMA analyse, wijzen de sterke uitwisseling van sediment poriënhouder en de bijdragen aan het chemisch karakter van de waterstroom erop dat het water-sediment grensvlak in het rivierbed een belangrijke rol speelt in de nitraatuitwisseling en de regulering in de waterstroom.

De EMMA methode is een goede en duidelijke benadering voor het vinden van bronnen van water en chemische stoffen. Bij deze methode kan een beperkt maar slim ontworpen ruimtelijk en temporeel bemonsteringsschema worden toegepast.

De meest beïnvloede kleine stroompjes en het sediment-water grensvlak stroomafwaarts van de afvalwaterzuiveringsinstallatie emissie in het kleine Pisões stroombekken bij Beja vertegenwoordigen belangrijke stroomlocaties voor stikstofverwijdering door processen zoals nitrificatie en denitrificatie. In sommige stroomsegmenten, en vooral die met significante ammoniumniveaus, sluiten wij niet uit dat er annamox reactiemechanismen optreden die de stikstofstromen beïnvloeden, samen met nitrificatie en denitrificatie. Kleine drainagekanalen en stroompjes, en vooral de water - sediment grensvlakken daarin, kunnen bij uitstek worden beschouwd als belangrijke locaties voor snelle nitrificatie en denitrificatie processen.

De analyse en interpretatie van de waterstof, zuurstof en stikstof isotopen van zowel water als nitraat is gedaan in combinatie met een zelf ontwikkelde “open source” modelformulering voor reactief transport. Deze benadering biedt goede mogelijkheden voor de analyse van het transport en het ‘‘lot’’ van voedingsstoffen in waterbekkens.
De bovenloopgebieden van de rivieren zijn geïdentificeerd als effectieve zones voor nitrificatie en denitrificatie, waarbij vrijgekomen overschotten aan ammonium van de afvalwaterzuiveringsinstallatie snel worden omgezet in nitraat, dat vervolgens uit het system wordt verwijderd door denitrificatieprocessen. Ook nitraat aanwezig in het ondiepe grondwater en dat opnieuw verschijnt in de grondwater uitstroom ondergaat een significante denitrificatie tijdens het transport en aan het sediment-water grensvlak. Door deze processen neemt de hoeveelheid nitraat benedenstrooms af, en verdwijnt bijna volledig wanneer het afvoerwater uit het stroomgebied het ontvangende Roxo water reservoir bereikt. Vandaar dat, in tegenstelling tot conventionele opvattingen, in dit geval van het Roxo reservoir, stikstof en nitraat gedurende bepaalde perioden van het jaar als beperkende factor voor het gebruik van voedingsstoffen optreden door bijvoorbeeld algen, terwijl er meestal een overschot is aan fosfaat dat kan worden geassocieerd met sediment in het water.
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