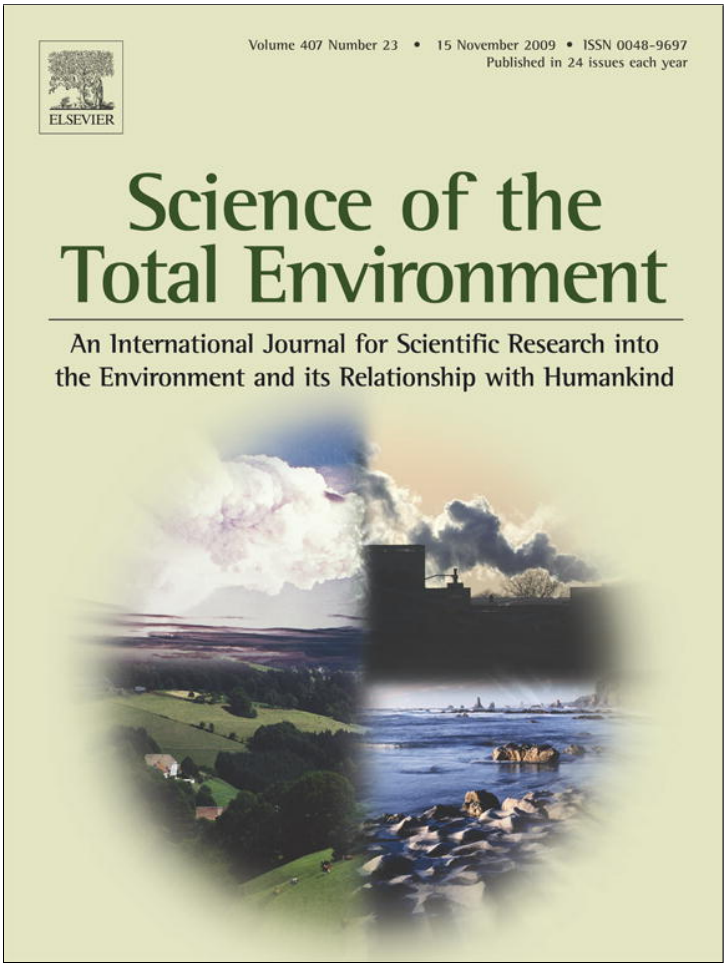
Volume 407 Number 23 • 15 November 2009 • ISSN 0048-9697
Published in 24 issues each year
ELSEVIER
Science of the Total Environment
An International Journal for Scientific Research into the Environment and its Relationship with Humankind

Science of the Total Environment 407 (2009) 6034–6043

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Agriculture-induced increase in nitrate concentrations in stream waters of a large Mediterranean catchment over 25 years (1981–2005)

Luis Lassaletta [a,*], Héctor García-Gómez [a], Benjamín S. Gimeno [b], José V. Rovira [a]

[a] Department of Ecology, Universidad Complutense de Madrid, c/José Antonio Novais s/n 28040 Madrid, Spain
[b] Ecotoxicology of Air Pollution, CIEMAT (ed. 70), Avda. Complutense 22, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 18 March 2009
Received in revised form 15 July 2009
Accepted 4 August 2009
Available online 8 September 2009

Keywords:
Nitrate levels
Temporal trends
Agricultural intensification
Land use
Water Framework Directive
Ebro River Basin

ABSTRACT

Anthropogenic activities influence past and present nitrate levels recorded in European stream waters, posing a threat to aquatic biota and human beings. Scarce information on temporal trends of nitrate concentration and its causes is available for Mediterranean catchments. This study presents the evolution of nitrate concentrations over 25 years in stream waters of the Ebro River Basin (Spain), a large Mediterranean catchment involving 85,566 km^2. Nitrate concentration increased with time in 46% of the 65 sites involved in the study. Agricultural cover of 30 hydrologically independent sub-catchments was the main land use related to nitrate concentration ($R^2=0.69$). Throughout the 25 year-period, the sites showing increased nitrate concentrations with time (trend sites) also presented an enhanced influence of agricultural cover on nitrate concentrations along the time frame of the study. As a result of these temporal changes, at the end of the studied period nitrate concentrations in stream waters responded similarly to agricultural cover in both trend and non-trend sites, showing non significant differences in the slope of the resultant regression models. At this time, agricultural cover explained 82% of the variability found in nitrate levels. If these trends remain unchanged, in 2015 many of the water bodies considered in this study would not comply with the requirements of the European Union Water Framework Directive (WFD). Therefore management decisions, mainly associated to agricultural practices, should be implemented as soon as possible at the catchment level to meet WFD objectives.

1. Introduction

Inland waters integrate terrestrial, atmospheric and aquatic processes and can be considered as sentinels of environmental changes (Williamson et al., 2008). Point and diffuse sources, such as human sewage, deposition of air pollutants and agricultural practices, induce an increase in nitrogen (N) loads in rivers, resulting in the cultural eutrophication of the environment (Camargo and Alonso, 2006). Several studies have analyzed temporal trends in N concentrations and fluxes of continental and coastal waters from North America and Europe. Heathwaite et al. (1996) reported increases in N concentrations in the rivers of these continents, reaching levels that were over 20 times higher than background levels. Similarly, Green et al. (2004) pointed out that global fluxes of dissolved inorganic nitrogen (DIN) in river basins all over the world have increased 6-fold since the pre-industrial time.

River catchments integrate the different factors influencing nutrient pathways to rivers. This fact has been acknowledged by the European Union (EU) Water Framework Directive (WFD, 60/2000/EC), which aims to improve the ecological condition of continental water bodies through environmental management initiatives designed at the catchment level. The WFD promotes the analysis of temporal trends in water quality and the identification of those European water bodies exceeding established reference nutrient levels. Contrasting results have been reported on the environmental benefits derived from other EU legislation initiatives related to water quality and the protection of continental aquatic ecosystems. Improvement of water treatment networks has enabled the reduction of phosphorus (P) and ammonium (NH_4^+) levels in many European river catchments. However, these initiatives have been less effective in controlling nitrate (NO_3^-) concentrations. This fact has become an issue of concern for the management and conservation of aquatic ecosystems (EEA, 2007a) as it could prevent achievement of the "good ecological status" of European rivers by the year 2015, which is the target of the WFD.

Although other chemical species associated to dissolved inorganic nitrogen (DIN), such as ammonium, can be predominant in some specific stream reaches, specially those near point sources, nitrate is considered as the major constituent of the total N pool in non-pristine rivers (Turner et al., 2003), from headwater streams (Peterson et al., 2001) to large rivers (Bernot and Dodds, 2005). In fact, increasing nitrate concentrations in fluvial waters is one of the main indicators of

* Corresponding author. Tel.: +34 91 394 4804; fax: +34 91 3945 081.
E-mail address: lassalet@bio.ucm.es (L. Lassaletta).

0048-9697/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.scitotenv.2009.08.002

the final stages of N saturation processes in watersheds (Stoddard, 1994; Fenn et al., 2008). In addition to the eutrophication of continental and coastal water bodies, nitrate can be toxic to freshwater animals and may induce adverse effects on human health. Camargo et al. (2005) pointed out that 44 mg/l of NO_3^- can adversely affect common freshwater species; the most sensitive ones could be affected by concentrations exceeding 8.8 mg/l of NO_3^-. International drinking water guidelines range 40–50 mg/l of NO_3^- to prevent human metahemoglobinemia. Although cancer and adverse reproductive outcomes have been associated to much lower concentrations, there is not scientific consensus on a suitable threshold related with human chronic exposure to nitrate (Ward et al., 2005).

Agricultural activities are the primary source of N pollution in European aquatic environments (EEA, 2005; Grizzetti et al., 2005). European agriculture has experienced a transformation from extensive to intensive practices during the last fifty years (EEA, 2007b). Although the amount of inorganic fertilisers applied per hectare has remained constant during the 1990–2002 period, the increasing application of organic N, with large regional variations in its supplementation rate across Europe, may induce a significant N export to water bodies (Oenema et al., 2007). Air pollution may also represent another significant N input into water bodies. Although nitrogen oxides (NO_x) and ammonia (NH_3) emissions and deposition have decreased in the EU during the 1990–2005 interval (EEA, 2007c; Fagerli and Aas, 2008), N critical loads are still exceeded in many European forests (Lorenz et al., 2008). This general scenario is similar in some Mediterranean countries of EU, such as Italy and Greece, where agricultural fertilization and air pollutant emissions have been reduced during the last decades (http://faostat.fao.org/; EEA 2007c).

Similarly to other EU countries, a progressive intensification of agriculture has occurred in the most productive areas of Spain since the beginning of "the Green Revolution" in 1960. As it is shown in Table 1, the main intensification indicators, such as consumption of fertilisers and pesticides, mechanization and irrigation, have grown since 1961 resulting in a steady increase of crop yields. Nevertheless, the most marginal fields have been abandoned during the same period (Varela-Ortega and Sumpsi, 2002). In addition, air pollution abatement policies have not been successfully implemented in Spain; in fact, NO_x and NH_3 emissions have increased by 22.4% and 18% in the 1990–2005 period (EEA, 2007c), and N concentration in rainfall has increased in northeastern Spain in the last 20 years (Ávila et al., in press). Similarly to other EU countries, the control of point source effluents has efficiently reduced P levels in Spanish rivers while it has been less successful in abating the concentration of N compounds (Ibáñez et al., 2008). These general trends together with the land use changes occurred in Spain may have influenced the N levels, especially nitrate concentrations, recorded in Spanish water bodies.

The detection of temporal trends in nitrate concentrations in Mediterranean rivers and their relationships with land use changes occurring at the catchment level contributes to identify the areas and factors adversely influencing water quality. Therefore it could be a useful tool for environmental managers. Some efforts have been made to study changes in water nutrient levels in the Mediterranean countries; however, they have not considered the influence of land use at catchment and sub-catchment levels. They mostly involve very specific sites, such as main channels and mouths of large rivers or sectors influenced by big cities or groundwater recharge (EEA, 2007a; Torrecilla et al., 2005; Ibáñez et al., 2008; Bouza-Deaño et al., 2008).

Table 1
Agricultural intensification indicators in Spain (1961–2005).

	1961–65	1981–85	2001–05
Agricultural area (10^3 km^2)	330	309	293
N fertilisers consumption (10^3 kg)	369	863	1064
Agricultural tractors (10^3 units)	111	591	953
Area equipped for irrigation (%)	6	10	13
Cereal yield (kg/ha)	1219	2144	3049

Source: http://faostat.fao.org/.

To cover this gap, in this study we explored the temporal trends (1981–2005) in nitrate concentrations in stream waters of the Ebro River Basin. The following hypothesis were contrasted: 1) Nitrate concentrations in surface waters increased over time, 2) land use, mainly agriculture, influenced nitrate levels in this period, and 3) this influence has changed with time. In addition, the implications of the results in relation to the achievement of WFD goals have been highlighted.

2. Study area

The study area involves the whole Ebro River Basin (NE Spain), 85,566 km^2, discharging into the western Mediterranean Sea (Fig. 1). It is the largest Spanish fluvial system, covering 17% of the Spanish Iberian territory. The main channel of the Ebro River represents 910 km of the total 13,049 km fluvial network. Average discharge of the Ebro River near its mouth was 9216 hm^3/y from 1981 to 2005. The catchment is heterogeneous in terms of geology, topography, and climatology. In general, silicic materials are located in the uppermost altitudes (3408 m a.s.l. is the highest altitude) while calcareous materials are found at lower elevations (1000–3000 m). The central part of the basin is an evaporitic Tertiary depression of marine origin with a thick layer of gypsum, halite and other salts. Topography modulates Mediterranean climatic patterns throughout the catchment, with a distinct transition from a semiarid environment in the centre of the catchment to humid conditions at its northern ranges, influenced by the Pyrenees. Average annual precipitation in the 1920–2002 period was 622 mm, varying from over 2000 mm in the Pyrenees to less than 400 mm in the arid interior (CHE, 2005).

About 3 million inhabitants live in the Ebro Basin, with a population density of *ca.* 34 inhabitants/km^2 (less than 50% of the Spanish mean). Around 45% of the population is concentrated in 5 towns over 100,000 inhabitants. The most populated area of the basin includes Zaragoza City and its surroundings, with more than 700,000 inhabitants. This area has undergone a significant industrial development during the last two decades (CHE, 2005). From 1981 to 2001, the population in the basin grew by 1%, and migrations from the least to the most populated towns occurred (http://www.ine.es/).

A great effort has been conducted in the catchment to control the quality of point source effluents. For instance, the number of wastewater treatment plants has increased from 1 to 259 from 1989 to 2005 (Oscoz et al., 2008). As a result, a drastic reduction in the urban inputs of nutrients has been found; in fact, Bouza-Deaño et al. (2008) and Ibáñez et al. (2008) reported reductions in phosphate (PO_4^{3-}) levels during that period. Torrecilla et al. (2005) estimated that point sources in the area of Zaragoza could account for 36% of all the nitrate inputs to the streams, while agricultural sources would explain the remaining percentage.

As shown in Table 2, agricultural and natural areas represent 48% and 50% of the total basin area, respectively (CORINE Land Cover Project data, 2000 data). Around 10% of the catchment is devoted to irrigated agriculture, demanding 6310 hm^3/y in the 90s, which represent 89% of the total water demand (CHE, 2005). Available data for Ebro River basin indicate that a great increase in water demand for irrigation (18%) occurred between 1961 and 1985, followed by a lower increase (8%) from 1985 to 2005 (Pinilla, 2006; CHE, 2005). Besides, during this last period the area devoted to maize, one of the most water-demanding crops, increased by 34%. Additionally, the yield of the main crops in the region raised substantially (17%, 21% and 25% for wheat, barley and grain maize, respectively) (http://ec.europa.eu/).

Annual averages of discharge through the 1981–2005 period were very variable among the Ebro tributaries in both reaches close to their sources (17–320 hm^3/y, 165 median value) and those near to their

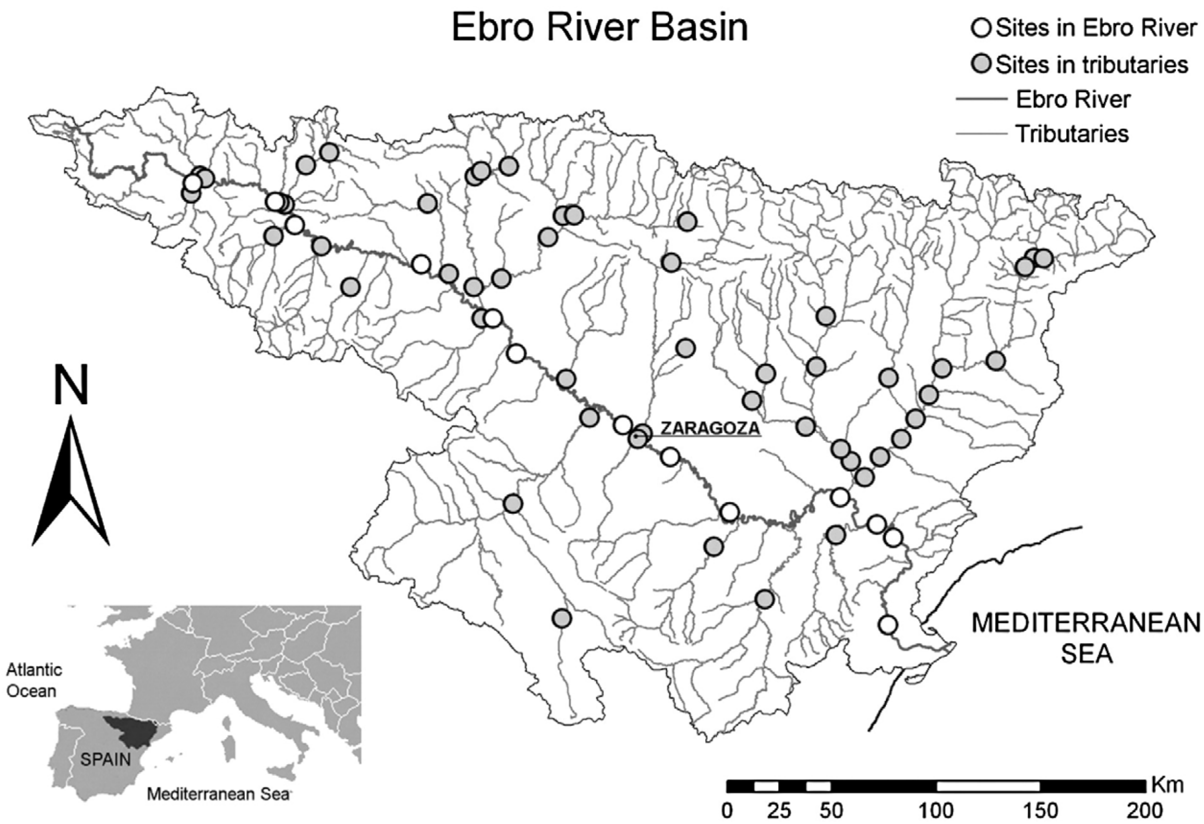

Fig. 1. Study area and location of the sites of the Ebro River catchment where nitrate concentrations were monitored.

mouths (91–1258 hm^3/y, 346 median value). Nitrate exports for the five Ebro sub-catchments where enough data were available were estimated following Négrel et al. (2007). The average nitrate export rates for 1981–2005, expressed as NO_3^-, ranged 500–2900 kg $km^{-2} y^{-1}$. According to worldwide study performed by Álvarez-Cobelas et al. (2008), these estimations correspond to catchments not extremely impacted by agriculture.

Table 2
CORINE Land Cover (CLC) classes and codes at the first and second levels, and their corresponding cover in the Ebro River catchment in 2000.

	Code	Class	Cover in the Ebro Basin[a]
First level	1	Artificial surface	1.09
	2	Agricultural areas	47.98
	3	Natural areas[b]	50.20
	4	Wetlands	0.09
	5	Water bodies	0.64
Second level	1.1	Urban	0.61
	1.2	Industrial/commercial[c]	0.34
	1.3	Mine/dump/construction	0.13
	1.4	Artificial green[d]	0.02
	2.1	Arable land	29.44
	2.2	Permanent crops	3.39
	2.3	Pastures	0.99
	2.4	Heterogeneous areas	14.16
	3.1	Forests	22.31
	3.2	Scrub/grasslands[e]	25.41
	3.3	Open spaces[f]	2.49
	4.1	Inland wetlands	0.05
	4.2	Maritime wetlands	0.04
	5.1	Inland waters	0.62
	5.2	Marine waters	0.02

[a] Expressed as percentage (%) of the whole catchment.
[b] Natural areas: *Forest and semi-natural areas.*
[c] Industrial/commercial: *Industrial, commercial and transport units.*
[d] Artificial green: *Artificial, non-agricultural vegetated areas*; this category involves recreational areas such as urban parks.
[e] Scrub/grasslands: *Scrub and/or herbaceous vegetation associations.*
[f] Open spaces: *Open spaces with little or no vegetation.*

3. Materials and methods

3.1. Nitrate concentration data

The Ebro Basin Confederation (*Confederación Hidrográfica del Ebro*: CHE)—the first water authority ever created and the first basin organization in the world—is, since its foundation in 1926, the institution in charge of the integrated basin-based water management of the Ebro River Basin (CHE, 2008a). Its Water Quality Control Network was progressively developed since the early 1960s. The Water Quality Department of CHE provided us a raw data-set of nitrate concentrations recorded from 1981 to 2005 at 65 sampling sites distributed all over the Ebro River Basin (Fig. 1). Nitrate was determined by UV–Visible Spectrophotometry until 1992 and by Ion Chromatography from then on. The detection limit for both methods was 0.1 mg/l of NO_3^-. Consistence of the data-set was tested and no biases in concentration data caused by this change were detected. These data correspond to surface water samples obtained periodically at fixed sites: 13 are situated in the main channel (Ebro River), 31 in the upper reaches of its tributaries (at a varying distance of 28–100 km from the tributary source), and 21 in the lower reaches (at a distance ranging 100–236 km from the tributary source). Sample collection, preservation and transportation were performed in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, American Public Health Association, 1992). The determination of nitrate concentrations was performed by the Water Quality Laboratory of the CHE always following good laboratory practices such as standard protocols, blank measurements, spiked and duplicated samples (APHA, 1992), and in accordance with the European EN 45001 Norm. The laboratory regularly participates in international programmes on quality control and it is currently accredited by UNE EN ISO/IEC 17025 Norm. In this work, nitrate values are expressed as mg/l of NO_3^-.

As varying sampling frequencies across sites and years were found in the raw data-set, periods with a more regular sampling were selected for consistency, following Helsel and Hirsch (2002). Therefore, the analyzed data-set involved the closest observations to January 15th and to July 15th, respectively labelled as "winter" and "summer" data.

3.2. Temporal trend analysis of nitrate concentrations

The non-parametric Seasonal Kendall test (SK test) (Hirsch et al., 1982) was performed to detect significant site-specific monotonic trends. The SK test can be used for time series of data with seasonal variations, missing values, tied values, or values below the limit of detection, and do not require data normality (Yu et al., 1993). The SK test applies the Mann–Kendall test (MK test) to each season separately, and then combines these results in an overall statistic. The slope of the significant summer and winter trends was estimated by using the Sen's slope procedure (Sen, 1968), while the slope of the overall significant trends was determined using the Seasonal Kendall trend slope method (SKT slope) (Helsel and Hirsch, 2002). Sen's slope is the median value of all slopes between every pair of data (in this case, winter or summer data) and SKT slope is a generalization of Sen's slope procedure, which avoids between-seasons biases. In summary, one "overall" and two "seasonal" site-specific trend analyses were considered, and the slope of the regression was estimated for every significant trend. The sites showing at least one significant trend were labelled as "trend sites"; those not showing any significant trend were labelled as "non-trend sites". We used an MS Excel® tool developed by Libiseller (2004) to perform the SK test; a similar tool was developed to estimate the slopes.

River flows in the Ebro Basin are regulated by reservoirs, diversions and consumptive uses, according to the management performed by local to national administrative agencies (Comín, 1999; Batalla et al., 2004). Therefore, following Hirsch et al. (1991) and Yu et al. (1993), nitrate concentrations were not flow-adjusted in our study because human activity has altered the probability distribution of discharge, through changes in regulation, diversion, or consumption during the period of the trend analysis.

Mean nitrate concentration for the overall studied period (1981–2005) was calculated for each site. In addition, average nitrate concentration values corresponding to the initial- (1981–1985) and final (2001–2005) periods were calculated and labelled as "initial concentration" and "final concentration", respectively. Comparison of these values between trend- and non-trend sites was carried out using the Student *t*-test; before performing the tests data were log-transformed to normalize data, normality was verified with the Shapiro–Wilk *W* test. For the trend sites, the relationship between the initial concentration (log-transformed) and the estimated SKT slope of the trend was analyzed using a General Linear Model (GLM) fitting.

3.3. Influence of land use on nitrate concentrations

The influence of land use on nitrate concentrations and trends was assessed only in hydrologically independent sub-catchments. Therefore, the closest sampling sites to the source of each tributary were selected, involving a total of 30 sites. The drainage areas upstream the sampling sites were delimited using spatial data layers (scale 1:50,000) provided by the CHE database (http://oph.chebro.es) and a GIS software (ArcGis 9.2). Fig. 2 shows the 30 resultant sub-catchments, with areas ranging from 223 km^2 to 3113 km^2 (1096 km^2, median value).

The land use maps from the CORINE Land Cover (CLC) Project were used to calculate the cover (%) of every land use class (first and second levels of CLC classification) in each sub-catchment for the years 1987 and 2000 (Table 2). Average value for these two years was calculated. Finally, net change in the cover of every CLC class during the 1987–2000 period was estimated by subtracting the percentage of expanded area from the percentage of lost area during that particular time span.

In order to evaluate whether differences in land uses existed between trend- and non-trend sites, mean contrasting tests (Student

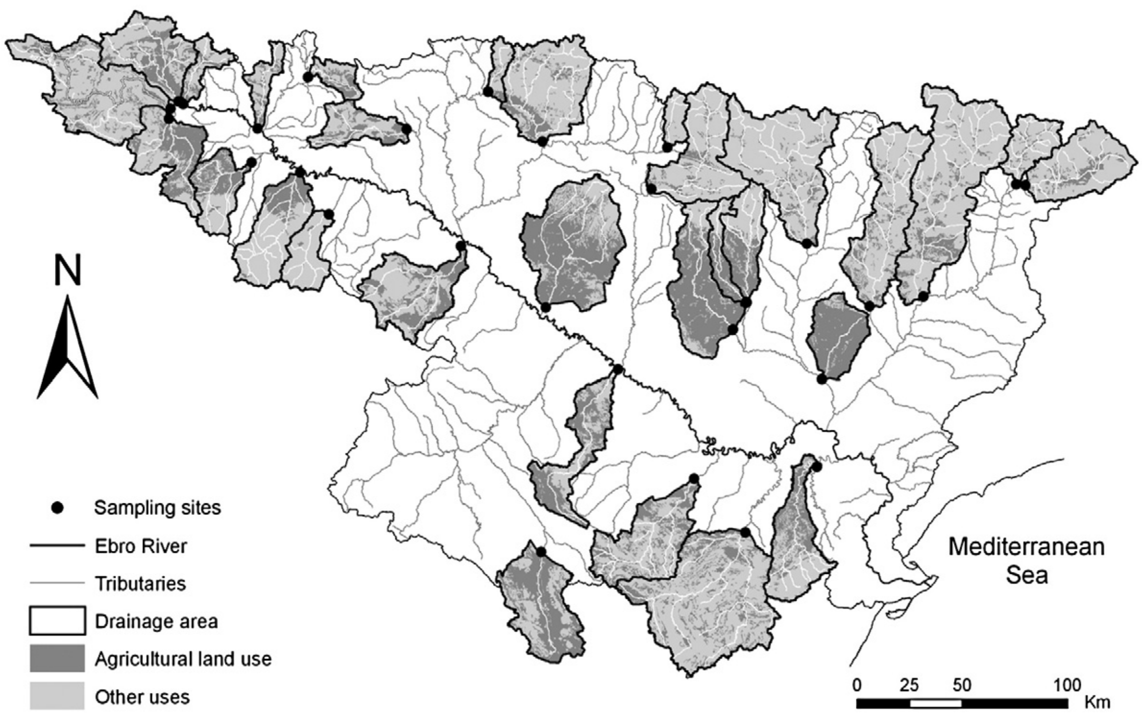

Fig. 2. Drainage areas of the 30 sites selected for the assessment of the influence of land uses on nitrate concentrations. Agricultural cover was obtained from CORINE Land Cover database (CLC, 2000).

t and Mann–Whitney *U* tests for normal and non-normal distributions, respectively) were performed for every CLC class at both 1st and 2nd levels. The variables involved in this comparison were average area between 1987 and 2000, and net change in the cover of the different CLC classes.

Two forward stepwise regressions were performed at each CLC level to obtain the CLC classes explaining the mean nitrate concentration for the overall period. Only "Agricultural areas" and its sub-classes were selected by the models. Nitrate concentration response to agricultural cover was studied by constructing five General Linear Models (GLM). The first GLM described the overall response of the mean nitrate concentration related to average agricultural cover throughout the studied period. Similarly, four additional GLM were constructed for each combination of trend- or non-trend sites and the initial or final nitrate concentrations. A parallelism test was performed in both pairs of models to test the homogeneity of their slopes. Nitrate concentration and percentage of agricultural land use areas were normalized by using log(x) and arcsin $[(y/100)^{1/2}]$ transformations, respectively.

The relationship between Sen's slope and the cover of the different CLC classes was studied by constructing new GLM. With the exception of trend analyses, all statistical analyses were performed using the computer programme STATISTICA 6.0 (StatSoft Inc., 2001), establishing 0.05 as the critical significance level.

3.4. Estimation of the compliance with the water framework directive

To place the results of our study in the context of the WFD and to assess whether the resulting data would comply with the WFD objectives, the initial, final, and estimated nitrate concentrations for 2015 were compared with two types of reference levels of NO_3^-: 1) 20 mg/l, which is a general threshold currently adopted by CHE (2008b) to achieve the Good Ecological Status of all the water body-types of the Ebro catchment (*unspecific-water body type threshold*), and 2) the maximum level of NO_3^- recorded at reference stations of each water body type of the Ebro catchment (Prat and Munné, 1999) (*specific-water body type threshold*). For trend sites, SKT slope was used to estimate future (2015) nitrate concentrations, whereas for non-trend sites, the mean nitrate concentration in the 1981–2005 period was used as the 2015 nitrate concentration value. This assessment involved the 52 sites that can be grouped according to the water body-types adopted by CHE (2005).

4. Results

4.1. Evolution of nitrate concentration

Nitrate was the dominant form of DIN, none of the sites presented an average molar ratio of nitrate/ammonium lower than 3.7, being 20.4 the median molar ratio for the 65 sites (Table 3). The average values of nitrate concentrations (1981–2005 period) were very variable among the 65 sites of the Ebro River Basin (1.3–40.3 mg/l; median = 9.4 mg/l; Table 3). All site-specific mean concentrations exceeded 0.44 mg/l of NO_3^-, the background concentration proposed by Meybeck (1982) for the major unpolluted rivers. Furthermore, 50% of them were greater than this background value by more than 21-fold. In addition, 58% of the sites overreached 8.8 mg/l, which is the threshold proposed by Camargo et al. (2005) for protecting the most sensitive freshwater species. The maximum allowable concentration established by the Nitrates Directive (1991/696/EC), 50 mg/l, was occasionally exceeded at some sites, but it was not exceeded when mean site values were considered.

In 48% of the sites ($n = 31$), a significant temporal trend was detected in at least one out of the three trend analyses performed: overall, winter or summer periods. Table 4 presents the number of sites showing a temporal trend in nitrate concentrations. 19 and 26 sites exhibited significant increases in winter and summer, respectively. Only one site exhibited a significant decrease (in summer). The trend sites are spread all over the Ebro catchment, mainly in the tributaries. Five sites located at the main channel of the Ebro River presented a significant increase in their nitrate concentrations (mainly in the seasonal periods). These sites were situated in the upper and lower sectors of the Ebro River but not in the mid-sector of the river. The correlation (Spearman test) between annual-averaged flow and time at the 39 sites where records of more than 15 complete years were available resulted in a significant ($p < 0.05$) flow decrease with time in 5 sites, 4 of them were non-trend sites and only one was a trend site.

When nitrate concentrations of the initial and final periods were compared, the non-outlier range (±1.5 SD) increased from 1.2–22.6 mg/l to 1.7–28.7 mg/l in the non-trend sites, and from 1.1–18.5 to 1.7–25.8 mg/l in the trend sites. In the initial period, summer mean nitrate concentrations were significantly higher in the non-trend sites than in the trend sites, whereas it did not differ between both site groups in the final period. Non significant differences were found between both types of sites in winter.

A significant linear relationship ($R^2 = 0.37$; $p < 0.01$) was found between the initial nitrate concentration and the SKT slope of the trend sites (Fig. 3). Similar results were observed in the relationships between the initial concentration and Sen's slope for seasonal data ($R^2 = 0.38$, $p < 0.01$ for winter values; $R^2 = 0.29$, $p < 0.01$ for summer values). Therefore, the trend sites with a higher initial concentration also had a higher estimated slope. Consequently, the increase in their nitrate concentration was faster with time.

4.2. Relationships between nitrate concentrations and land use

A significant trend in nitrate concentration was detected in 11 of the 30 hydrologically independent sites in at least one of the considered time frames (i.e., winter, summer or overall period). The only decreasing trend was found at a site belonging to the sub-catchment showing the highest and the lowest cover by the CLC "Natural areas" and "Agricultural areas", 97% and 2% respectively.

Small changes were found in the cover of the different CLC classes from 1987 to 2000 in the 30 sub-catchments (Table 5). The greatest average net change was 0.6% ($n = 30$) and none of the CLC classes experienced a net change over 5% of the area of any sub-catchment. Mean net change of agricultural land was *ca.* −0.1%, which suggests an agricultural abandonment much lower than for other regions of the Iberian Peninsula (Table 1). Significant differences ($p < 0.05$) in mean

Table 3
Averages of nitrate concentrations (expressed as mg/l of NO_3^-) and molar ratio of nitrate/ammonium recorded during 1981–2005 at the 65 sites of the Ebro River catchment involved in the study.

	Mean	Minimum	1st quartile	Median	3rd quartile	Maximum	Std. Dev	Abs. max.[a]
Winter	11.4	1.5	5.3	11	15.3	41.6	7.3	65.1
Summer	9.6	1.2	3.9	8.2	12.4	39.1	7.1	64.6
Overall	10.5	1.3	4.5	9.4	14.5	40.3	6.9	65.1
NO_3^-/NH_4^+ molar ratio	22.6	3.7	8.8	20.4	32.0	60.6	15.4	249.1

[a] Absolute maximum value for the raw data-set. Number of observations (n): $n_{summer} = 1560$; $n_{winter} = 1503$; $n_{total} = 3063$.

Table 4
Number of sites within the Ebro catchment experiencing significant changes in nitrate concentration with time (1981–2005), classified according to their location and their type of trend.

Trend		Tributaries		Main channel[c]	Total
		Upper reaches[a]	Lower reaches[b]		
Winter	No trend[d]	21	14	11	46
	Increase	10	7	2	19
	Decrease	0	0	0	0
Summer	No trend	17	11	10	38
	Increase	13	10	3	26
	Decrease	1	0	0	1
Overall	No trend	19	11	12	42
	Increase	12	10	1	23
	Decrease	0	0	0	0
Groups	Non-trend sites[e]	15	11	8	34
	Trend sites[f]	16	10	5	31
	Total	31	21	13	65

[a] *Upper reaches:* sites located at a distance of 28–100 km from the tributary source.
[b] *Lower reaches:* sites located at a distance of 100–236 km from the tributary source.
[c] *Main channel:* sites located in the Ebro River.
[d] *No trend:* sites not presenting significant temporal trends in nitrate concentrations.
[e] *Non-trend sites*: sites where no significant trends were found at any time frame (winter, summer or overall).
[f] *Trend sites:* sites exhibiting significant temporal trends for nitrate concentrations at any time frame (winter, summer or overall).

net changes between trend sites sub-catchments and non-trend sites ones were found only for two classes: 1.2 class “Industrial/commercial” and 1.4 class “Artificial green”. Low increases in the cover of these two classes occurred in the sub-catchments of non-trend sites whereas no changes were found in the sub-catchments of trend sites. Conversion from dry to irrigated arable land occurred in 10 of the 30 sub-catchments, involving 4 trend sites and 6 non-trend sites. These changes in sub-catchment area averaged *ca.* 1.4% for both types of sub-catchments.

As shown in Table 6, hardly any significant difference was found in the average area (1987 and 2000) between trend- and non-trend sites. Only “Artificial green” and “Scrub/grasslands” classes were significantly lower ($p<0.05$) in the trend sites when compared to the non-trend sites: 0% *vs.* 0.02% for “Artificial green”, and 24% *vs.* 33% for “Scrub/grasslands”, respectively.

Nitrate mean concentration (1981–2005 period) was strongly related to the average cover of “Agricultural areas” ($R^2=0.69$; $p<0.001$) (Fig. 4). This relationship was also evaluated for trend- and non-trend sites using the nitrate average concentrations recorded

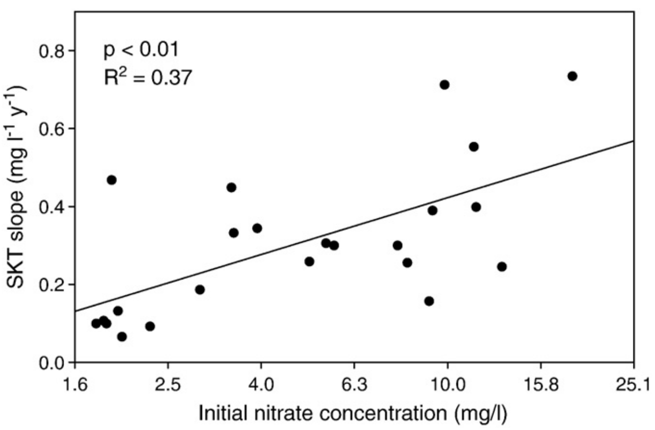

Fig. 3. Relationship between the initial concentration of nitrate (expressed as mg/l of NO_3^-) in the Ebro River catchment (1981–1985 period) and the estimated slope of the temporal trend. To improve the understanding of the figure, the log-transformed data of nitrate concentrations (*x*-axis) are shown in their original units.

Table 5
Net changes in the area covered by each CORINE Land Cover class at 30 hydrologically independent sub-catchments of the Ebro River Basin occurring between 1987 and 2000.

CLC class		Net changes[a]			
		Mean	Range		Trend- vs non-trend sites[b]
First level	1 Artificial surface	0.14	−0.05	1.01	n.s.
	2 Agricultural areas	−0.08	−0.84	0.61	n.s.
	3 Natural areas	−0.08	−0.72	0.17	n.s.
	4 Wetlands	0.00	−0.01	0.00	n.s.
	5 Water bodies	0.02	0.00	0.28	n.s.
Second level	1.1 Urban	0.04	0.00	0.51	n.s.
	1.2 Industrial/ commercial	0.07	0.00	0.87	*
	1.3 Mine/dump/ construction	0.02	−0.32	0.31	n.s.
	1.4 Artificial green	0.01	0.00	0.10	*
	2.1 Arable land	−0.14	−1.28	0.43	n.s.
	2.2 Permanent crops	0.07	−0.59	1.48	n.s.
	2.3 Pastures	−0.01	−0.39	0.10	n.s.
	2.4 Heterogeneous areas	0.00	−1.13	0.60	n.s.
	3.1 Forests	0.61	−2.95	4.21	n.s.
	3.2 Scrub/grasslands	−0.42	−4.59	3.78	n.s.
	3.3 Open spaces	−0.27	−2.04	0.03	n.s.

The second level classes corresponding to first level classes *Wetlands surfaces* and *Water surfaces* are not further displayed, as they are conformed by only one subclass each: *Inland wetlands* and *Inland waters*, respectively.
[a] Numeric values are expressed as percentage of sub-catchment area; negative values correspond to net losses and positives ones to net gains.
[b] Mean comparison between trend sites ($n=11$) and non-trend sites ($n=19$). * $p<0.05$; n.s. non significant differences.

at the initial and final intervals, resulting in statistically significant relationships (Fig. 5). For the initial period, the regression involving the non-trend sites explained more variance and its slope was higher than for the trend sites. However, for the final period, there was no difference in R^2 or in the slope between these two groups. This result was confirmed by the parallelism test of those regression models, which identified significant heterogeneous slopes in the initial period

Table 6
Comparison of the surface area covered by each CORINE Land Cover class[a] (1987–2000 average) at trend- and non-trend sites from the 30 hydrologically independent sub-catchments of the Ebro River Basin.

CLC class	Trend-sites			Non-trend sites			Differences[b]
	Mean	Min.	Max.	Mean	Min.	Max.	
1 Artificial surface	0.7	0.1	2.7	0.8	0.2	1.9	n.s.
2 Agricultural areas	37.6	2.2	91.2	32.7	9.2	74.4	n.s.
3 Natural areas	61.5	7.7	97.1	65.7	24.3	89.3	n.s.
4 Wetlands	<0.1[c]	0.0	0.2	<0.1	0.0	0.2	n.s.
5 Water bodies	0.1	0.0	0.4	0.8	0.0	5.4	n.s.
1.1 Urban	0.4	0.1	0.7	0.5	0.1	1.5	n.s.
1.2 Industrial/ commercial	0.2	0.0	2.0	0.1	0.0	0.5	n.s.
1.3 Mine/dump/ construction	0.1	0.0	0.3	0.2	0.0	1.3	n.s.
1.4 Artificial green	0.0[c]	0.0	0.0	<0.1	0.0	0.1	*
2.1 Arable land	25.3	0.6	73.9	22.2	3.7	70.6	n.s.
2.2 Permanent crops	0.8	0.0	4.0	1.1	0.0	12.7	n.s.
2.3 Pastures	1.4	0.0	7.9	2.0	0.0	7.9	n.s.
2.4 Heterogeneous areas	10.1	1.5	34.6	7.4	0.0	21.6	n.s.
3.1 Forests	34.4	2.1	65.0	29.20	8.7	63.5	n.s.
3.2 Scrub/grasslands	23.6	5.5	39.1	33.1	15.3	49.1	*
3.3 Open spaces	3.5	0.0	18.4	3.4	0.0	16.1	n.s.

The second level classes corresponding to first level classes *Wetlands surfaces* and *Water surfaces* are not further displayed, as they are conformed by only one subclass each: *Inland wetlands* and *Inland waters*, respectively.
[a] Expressed as percentage of sub-catchment area.
[b] Comparison between trend sites ($n=11$) and non-trend sites ($n=19$). * $p<0.05$; n.s. non significant differences.
[c] 0.0 means no presence of this use in the catchment, <0.1 means a percentage between 0 and 0.1.

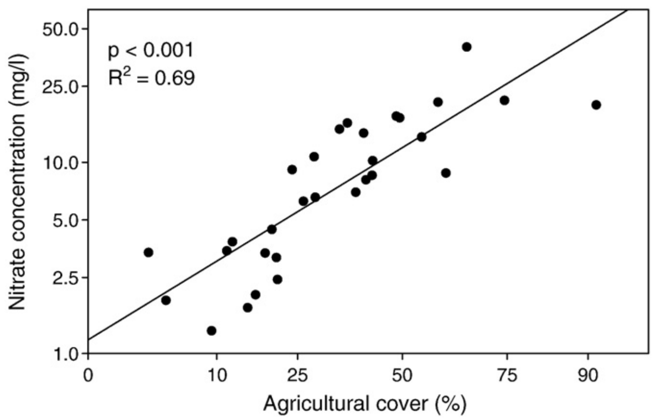

Fig. 4. Relationship between agricultural cover (%) in each sub-catchment and nitrate mean concentration (expressed as mg/l of NO_3^-) for the period 1981–2005. The analysis involved the 30 sites from the hydrologically independent sub-catchments. Data related with agricultural cover and nitrate concentrations were transformed, as arcsin $[(x/100)^{1/2}]$ and $\log(y)$, respectively. Data are shown in their original units to improve the understanding of the figure.

($p<0.01$) and homogeneous slopes in the final period. Consequently, at the end of the studied period the relationship between "Agricultural areas" and nitrate concentration in trend sites sub-catchments became similar to the corresponding relationship for non-trend sites sub-catchments, as their regression models had a similar explicative value and a similar slope.

In both winter and summer, Sen's slope was positively related with "Agricultural areas" cover, and negatively with "Natural areas" (Table 7). Thus, sub-catchments with higher agricultural cover, and consequently with lower natural cover, experienced a faster increase in their nitrate concentrations during the last 25 years. When considering the second level of CLC, it became apparent that the relationships found in winter and summer for the first level of CLC were related to the "Arable land" category. Additionally, negative relationships were found in summer between "Forests" or "Open spaces" and Sen's slope.

4.3. Analysis of compliance with the water framework directive

In the initial period, the unspecific-water body type nitrate concentration threshold (20 mg/l) was exceeded in 12% of the sites, while the specific-water body type thresholds were overreached in 35% of the sites (Table 8). In the final period, non-compliance with these thresholds raised to 17% and 52% of the sites, respectively. If the trend sites steadily increase their nitrate concentrations at the rate estimated in this study, 23% of the sites will exceed the unspecific-water body type threshold in 2015 and 56% will overreach the specific-water body type thresholds.

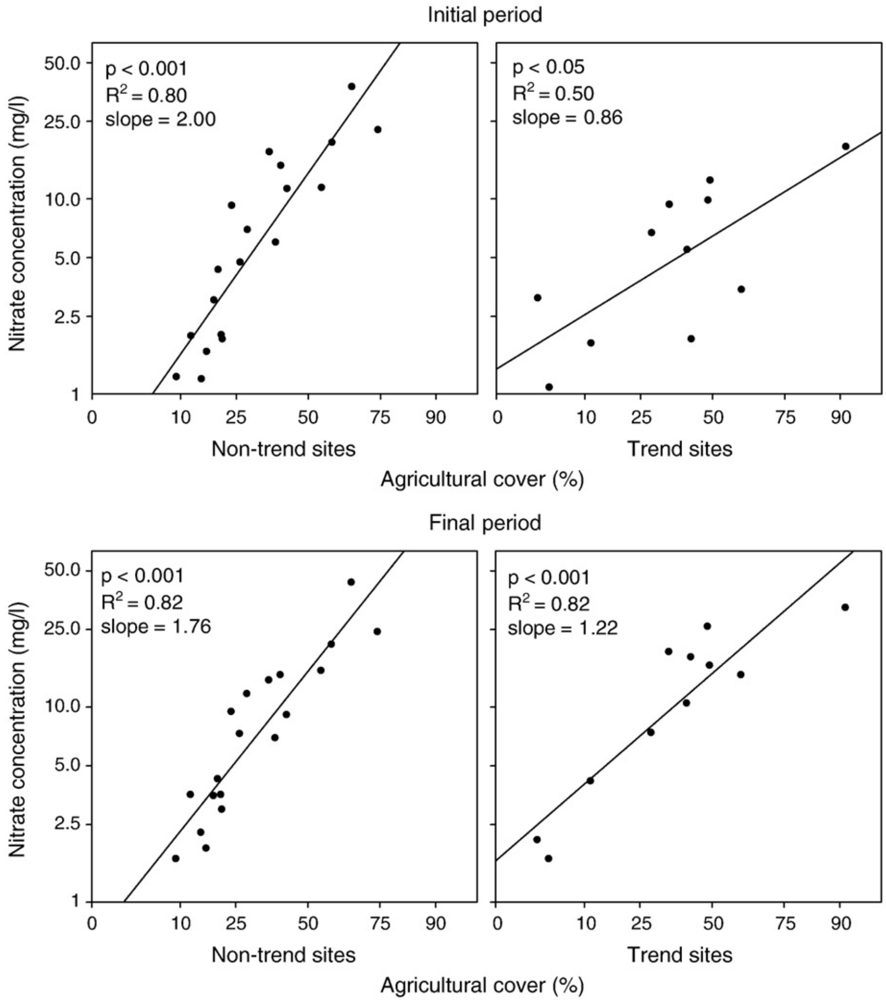

Fig. 5. Relationship between agricultural cover (%) in each sub-catchment and nitrate mean concentration (expressed as mg/l of NO_3^-) at the initial (1981–1985) and final (2001–2005) periods. The analysis involved the 30 sites from the hydrologically independent sub-catchments, grouped in trend- and non-trend sites ($n=11$ and $n=19$, respectively). Data related with agricultural cover and nitrate concentrations were transformed, as arcsin$[(x/100)^{1/2}]$ and $\log(y)$, respectively. Data are shown in their original units to improve the understanding of the figure.

Table 7
Results of the regression analysis relating the area covered by a given CLC class (1987–2000 average) and the estimated Sen's slope of the trend analysis involving the 30 hydrologically independent sub-catchments.

	Code	Class	Winter			Summer		
			p-value	R^2	Δ[a]	*p*-value	R^2	Δ
Level 1	1	Artificial surface	n.s.			n.s.		
	2	Agricultural areas	0.020	0.56	+	0.007	0.67	+
	3	Natural areas	0.021	0.55	−	0.008	0.66	−
	4	Wetlands	n.s.			n.s.		
	5	Water bodies	n.s.			n.s.		
Level 2	1.1	Urban	n.s.			n.s.		
	1.2	Industrial/ commercial	n.s.			n.s.		
	1.3	Mine/dump/ construction	n.s.			n.s.		
	1.4	Artificial green	n.s.			n.s.		
	2.1	Arable land	0.034	0.49	+	0.010	0.63	+
	2.2	Permanent crops	n.s.			n.s.		
	2.3	Pastures	n.s.			n.s.		
	2.4	Heterogeneous areas	n.s.			n.s.		
	3.1	Forests	n.s.			0.010	0.59	−
	3.2	Scrub/grasslands	n.s.			n.s.		
	3.3	Open spaces	n.s.			0.009	0.64	−

[a] Sign of the relation.

5. Discussion

5.1. Nitrate concentrations, temporal trends and agricultural practices

The increase in nitrate concentrations found in 46% of the sites involved in this study during the 1981–2005 period contrasts with the trends recorded in the fluvial waters of other European catchments where stabilization or reductions in nitrate concentration have been reported (EEA, 2003). Stabilization of nitrate concentrations has been observed in forested sites (Skjelkvåle et al., 2005) and agricultural catchments (Bechmann et al., 2008) of northern Europe. Reductions in other catchments have been attributed to the improvements in agricultural practices and to the reduction of fertiliser application or the abandonment of agricultural areas in Estonia (Iital et al., 2005, Järvet et al., 2002), the implementation and improvements of wastewater treatments in Poland and France (Eriksson et al., 2007; Tudesque et al., 2008), or the reduction of acid deposition in Czech Republic (Oulehle et al., 2008). There are exceptions to this general scenario in some Italian forests (Rogora, 2007) or agricultural catchments from northern Europe and France (Räike et al., 2003; Sileika et al., 2006; EEA, 2007a), in which N inputs have an atmospheric or fertiliser origin, where increasing trends of nitrate concentrations with time have been reported.

The results of this study indicate that agricultural cover strongly influences the average level of nitrate in the stream waters of the Ebro River catchment, explaining 69% of the variance over the 25-year period. A strong association between nitrate concentration and agricultural cover also has been found in other European catchments, accounting for a significative percentage (40%–90%) of the variance (Moreno et al., 2006; Skoulikidis et al., 2006; García-Pintado et al., 2007; Lassaletta, 2007; Tisseuil et al., 2008). Moreover, a convergence with time was found in the relationship between agricultural cover and nitrate concentrations in trend- and non-trend sites of the Ebro catchment over the 25-year period. The components explaining this convergence were: 1) the explained variance of the regression model of the trend sites converged with the corresponding value of the non-trend sites model, accounting for 82% of the total variance in the final period, and 2) the resulting slopes of the models constructed for the trend- and the non-trend sites were different at the initial period, becoming similar at the final period. This convergence was caused by the faster increase in nitrate concentration in those trend sub-catchments having a higher agricultural cover, as shown by the significant relationship between the Sen's slope and the latter variable.

As it is shown in Table 1, intensification of agriculture in Spain began before 1981, and it continued throughout the study period (1981–2005). The strong relationship between agricultural cover and nitrate concentration in non-trend sites in the initial period (1981–1985) suggests that the response to intensification probably started before 1981. Changes in nitrate concentration since the 1950s have been described in studies conducted in other European catchments (EEA, 2003; Radach and Patsch, 2007; Howden and Burt, 2008). Moreover, Billen et al. (2007) suggested that divergence from pristine conditions would have started several centuries ago.

As agricultural cover was similar in trend- and non-trend sites and it showed no significant changes with time, the temporal increase of nitrate levels could be associated to a delayed response of trend sub-basins to the general process of agricultural intensification that occurred in the Ebro basin. The different response velocities between sites might be explained by longer water-transit times, different stages of the saturation processes or differences in the implementation of newer agricultural practices in trend sites. Differences in time lag between N input and output has been observed in other European catchments (Sileika et al., 2006; Howden and Burt, 2008).

In non-trend sites nitrate levels remained unchanged over time and showed the same response to agricultural cover during the studied period. This fact could indicate that the response of their surface waters to agriculture reached a steady-state, which induces a stabilization in nitrate concentrations as has been found by Howden and Burt (2008) in other agricultural catchments. However, this could

Table 8
Percentage of water bodies exceeding the thresholds of nitrate concentration (expressed as mg/l of NO_3^-) for good ecological status in 1981–1985, 2001–2005 and 2015.

Type of water body[a]	Nitrate thresholds[b] (mg/l)	No. of sites	Sites at risk (%)[c]		
			1981–1985 (average)	2001–2005 (average)	2015[d] (estimated)
Mediterranean mineralized mountain streams at low altitude	10.5	8	63	100	100
Mediterranean mountain streams in calcareous terrains	21	11	0	9	9
Low-mineralized continental Mediterranean rivers	9	15	47	73	73
High-mineralized continental Mediterranean rivers	10.5	2	100	100	100
Large Mediterranean watercourses	19	6	17	17	17
Humid mountain streams in calcareous terrains	5.5	10	30	40	60
Total		52	35	52	56
All types	20[e]	52	12	17	23

Sites located in artificial or heavily modified water bodies (substantially changed as a result of physical alterations by human activity) were not considered in this part of the study ($n = 13$).
[a] Types according to CHE (2005).
[b] Specific-water body type thresholds, based on Prat and Munné (1999).
[c] Percentage of water bodies in which nitrate levels are above the indicated threshold.
[d] The estimation for 2015 was obtained through the SKT slope for the 21 sites with a significant overall trend, and whole-period means were used as estimators for the other 31 sites.
[e] Unspecific-water body type threshold: currently adopted by CHE (2008b); it is independent from typology.

be a transient stage as changes in factors mainly related with basin management could occur in the future. The convergence with time of trend sites to non-trend sites in the response to agriculture could not necessarily indicate that a steady-state has been also reached.

The increases in summer and winter nitrate concentrations with time observed at the catchment level could be explained by the type of agricultural practices performed in the sub-catchments. On the one hand, non-irrigated fields could produce the maximum export and concentration of this molecule in winter, when fertilization is carried out, precipitation is higher and plant nitrogen uptake is lower (Lassaletta, 2007; Tisseuil et al., 2008). On the other hand, irrigated agriculture induces nitrate inputs to stream waters through the irrigation return flows in summer (Torrecilla et al., 2005; Causapé et al., 2004).

The remaining unexplained 18% of the variance of nitrate concentrations recorded in the catchment in the final period may be attributable to other factors such us atmospheric N deposition, point sources or local environmental features of sub-catchments. In Spain, NO_x and NH_3 emissions have increased during the last years. In fact, if the present trend is maintained, by the year 2010 NO_x emissions will exceed by 50% the Spanish ceiling established by the EU National Emission Ceilings Directive (NEC Directive) (EEA, 2008). However, the high nitrate levels in aquatic systems associated with agricultural practices will probably mask the effects of atmospheric N deposition. In European forested catchments where the responses to N deposition have been studied (Rogora, 2007; Oulehle et al., 2008), the nitrate concentrations in stream waters (0.13–7.9 mg/l of nitrate) were generally lower than those measured in the Ebro sites (1.3–41.4 mg/l of nitrate, 9.2 median value). In the specific sub-catchment where a decreasing trend in nitrate concentration with time was found, agricultural land cover class was hardly represented, as 97% of its area was covered by the "Natural areas" CLC class. Therefore, it can be concluded that N deposition has not induced a clear increase in nitrate stream concentrations. Total atmospheric N deposition recorded at the nearest EMEP station (EMEP-Logroño 42º 27′N; 02º 21′W) remained in the range of 5–13 kg N ha^{-1} y^{-1} in the 1988–2000. According to Dise et al. (2009), it is unlikely that N values within this range could result in a relevant increase in nitrate concentrations in stream waters, especially when they are compared with agricultural inputs. However, N deposition may contribute to an acceleration of N saturation symptoms in those sub-catchments already impacted by large inputs from agricultural activities.

Population has hardly changed during the studied period and wastewater treatment has been improved. The only significant decreasing trend in nitrate was observed in summer in a sub-catchment with a very low agricultural influence (2%), which did not show any changes in N atmospheric deposition, in population, or in land uses. Impact of N point sources on river water quality is frequently higher during summer, influenced by lower stream flows. The improvement of depuration systems throughout the studied period can explain this decline in nitrate levels. However, the European objectives related with water depuration have not been achieved yet. This situation could aggravate the effects derived from agricultural pollution as the summation of point and diffuse source pollution may overwhelm stream capacity to retain and transform nutrients (Merseburger et al., 2005).

5.2. Ecological status of the stream water bodies and compliance with the WFD

The results of our study indicate that if control policies are not implemented, a substantial fraction of the stream water bodies of the Ebro River Basin will not achieve the WFD objectives in 2015, especially in those areas presenting a high agricultural cover. The positive relationship found between the increasing trend slope and the initial nitrate concentration suggests the need in prioritizing the control over the most impacted sub-catchments, not only because of their lower water quality, but also due to their faster deterioration.

Codes of good agricultural practices must be applied at the catchment level. Other approaches, such as stream and riparian restoration strategies, should be promoted as well (Cherry et al., 2008). Some programmes have been successful in minimizing the agricultural impacts on stream nitrate levels in Estonia (Iital et al., 2005) or Denmark (Kronvang et al., 2008). These programmes include reductions or control of fertiliser applications, improvements in farm management practices or regulations on point source discharges from wastewater treatment plants. In other cases, only small improvements have been observed in The Netherlands (Oenema et al, 2005), Poland (Eriksson et al., 2007) or Norway (Bechmann et al., 2008). The lack of an immediate response to the implementation of mitigation methods could be caused by the chronic accumulation of N in soils, implying that N surpluses would be continuously exported for years (Sileika et al., 2006; Cherry et al., 2008; Tisseuil et al., 2008). Therefore, control policies should be implemented as soon as possible in the Ebro River Basin in order to achieve the WFD objectives by 2015. Furthermore, new studies should be performed in the future to assess the evolution of nitrate concentration in this basin once mitigation policies have been implemented.

6. Conclusions

During the studied period (1981–2005), 46% of the monitored sites of the Ebro River Basin experienced increases in nitrate concentrations. Agricultural practices were the main factor explaining the observed patterns of nitrate concentrations. Agricultural cover explained 69% of the variability of nitrate concentration throughout the sub-catchments and the studied period. However two types of responses with time were found. Most sub-catchments showed an unchanged relationship, while those presenting temporal trends showed an increased response of nitrate concentrations to agricultural cover. As a result, a convergence towards a homogenization of the response of nitrate concentration to agricultural cover was found for the overall catchment. Thus, agricultural cover explained 82% of the variance of nitrate concentrations at the beginning of the 21st century. As the cover of the different land use types remained almost constant throughout the studied period, agriculture intensification is the most likely factor explaining the observed temporal patterns. The response of nitrate to this intensification probably occurred prior to 1981 in non-trend sites.

If present trends remain unchanged until 2015, 23% of the sites involved in this study would exceed the unspecific CHE threshold of 20 mg/l of NO_3^-, and 56% of them would overreach the specific-water body type thresholds considered in this work. Because it is likely that there will be a delay in the response of water quality to the management actions that could be implemented, the immediate control of agricultural non-point pollution is an urgent task in order to achieve the WFD goals in the Ebro River Basin.

Acknowledgements

This research was carried out under the framework of the European Science Foundation (ESF) project "Nitrogen in Europe" (NinE). We greatly acknowledge the Ebro Basin Confederation for providing nitrate data and relevant information on data quality. This research was funded by an agreement between the Spanish Ministry of Environment Rural and Marine Affairs (Ministerio de Medio Ambiente, Rural y Marino), CIEMAT, and the Universidad Complutense de Madrid on "Critical loads and levels". We are particularly grateful to Rocío Alonso and Ignacio Gonzálex-Fenández for their valuable inputs. We appreciate the comments of four anonymous reviewers which contributed to a substantial improvement of the quality of the revised manuscript.

References

Álvarez-Cobelas M, Angeler DG, Sánchez-Carrillo S. Export of nitrogen from catchments: a worldwide analysis. Environ Poll 2008;156:261–9.

APHA (American Public Health Association). In: Greenberg AE, Clesceri LS, Eaton AD, editors. Standard methods for the examination of water and wastewater. 18th Ed. Washington, DC: APHA-AWWA-WEF; 1992. p. 1100.

Ávila A, Molowny-Horas R, Gimeno BS, Peñuelas, J. Analysis of decadal time series in wet N concentrations at five rural sites in NE Spain. Water Air Soil Pollut in press. doi:10.1007/s11270-009-0124-7.

Batalla RJ, Gómez CM, Mathias-Kondolf G. Reservoir-induced hydrological changes in the Ebro River basin (NE Spain). J Hydrol 2004;209:117–36.

Bechmann M, Deelstra J, Stålnacke P, Eggestad HO, Øygarden L, Pengerud A. Monitoring catchment scale agricultural pollution in Norway: policy instruments, implementation of mitigation methods and trends in nutrient and sediment losses. Environ Sci Policy 2008;11:102–14.

Bernot M, Dodds WK. Nitrogen retention, removal, and saturation in lotic ecosystems. Ecosystems 2005;8:442–53.

Billen G, Garnier J, Némery J, Sebilo M, Sferratore A, Barles S, et al. A long-term view of nutrient transfers through the Seine river continuum. Sci Total Environ 2007;375: 80–97.

Bouza-Deaño R, Ternero-Rodríguez M, Fernández-Espinosa AJ. Trend study and assessment of surface water quality in the Ebro River (Spain). J Hydrol 2008;361:227–39.

Camargo JA, Alonso A. Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: a global assessment. Environ Int 2006;32:831–49.

Camargo JA, Alonso A, Salamanca A. Nitrate toxicity to aquatic animals: a review with new data for freshwater invertebrates. Chemosphere 2005;58:1255–67.

Cherry KA, Shepherd M, Withers PJA, Mooney SJ. Assessing the effectiveness of actions to mitigate nutrient loss from agriculture: a review of methods. Sci Total Environ 2008;406:1-23.

Causapé J, Quílez D, Aragués R. Salt and nitrate concentrations in the surface waters of the CR-V irrigation district (Bardenas I, Spain): diagnosis and prescriptions for reducing off-site contamination. J Hydrol 2004;295:87-100.

CHE. Caracterización de la demarcación y registro de zonas protegidas. Zaragoza: Confederación Hidrográfica del Ebro; 2005. p. 246.

CHE. River basin organisations in the 21st century. Zaragoza: Confederación Hidrográfica del Ebro; 2008a. p. 76.

CHE. Control del estado de las masas de agua superficiales. Informe de situación. Zaragoza: Confederación Hidrográfica del Ebro; 2008b. p. 186.

Comín FA. Management of the Ebro river basin: past, present and future. Wat Sci Tech 1999;40:161–8.

Dise NB, Rothwell JJ, Gauci V, van der Salm C, de Vries W. Predicting dissolved inorganic nitrogen in European forests using two independent databases. Sci Total Environ 2009;407:1798–808.

EEA. Europe's water: an indicator based assessment. Topic report No 1/2003. Copenhagen: European Environmental Agency; 2003. p. 97.

EEA. Source apportionment of nitrogen and phosphorus inputs into the aquatic environment. EEA report/No 7 2005. Copenhagen: European Environmental Agency; 2005. p. 48.

EEA. Assessing water quality in Europe using stratification techniques. Results of a prototype application using French data. Copenhagen: European Environmental Agency; 2007a. p. 73.

EEA. Europe's environment. The fourth assessment. EEA report No 1 2007. Copenhagen: European Environmental Agency; 2007b. p. 452.

EEA. Annual European community LRTAP convention emission inventory report 1990–2005. Copenhagen: European Environmental Agency; 2007c. p. 87.

EEA. NEC Directive status report 2007. EEA technical report No 9/2008. Copenhagen: European Environmental Agency; 2008. p. 41.

Eriksson H, Pastuszak M, Löfgren S, Mörth C-M, Humborg C. Nitrogen budgets of the Polish agriculture 1960–2000: implications for riverine nitrogen loads to the Baltic Sea from transitional countries. Biogeochemistry 2007;85:153–68.

Fagerli H, Aas W. Trends of nitrogen in air and precipitation: model results and observations at EMEP sites in Europe, 1980–2003. Environ Poll 2008;154:448–61.

Fenn ME, Jovan S, Yuan F, Geiser L, Meixner T, Gimeno BS. Empirical and simulated critical loads for nitrogen deposition in California mixed conifer forests. Environ Poll 2008;155:492–511.

García-Pintado J, Martínez-Mena M, Barbera GG, Albaladejo J, Castillo VM. Anthropogenic nutrient sources and loads from a Mediterranean catchment into a coastal lagoon: Mar Menor, Spain. Sci Total Environ 2007;373:220–39.

Green PA, Vörösmarty CJ, Meybeck M, Galloway JN, Peterson BJ, Boyer EW. Pre-industrial and contemporary fluxes of nitrogen through rivers: a global assessment based on typology. Biogeochemistry 2004;68:71-105.

Grizzetti B, Bouraoui F, de Marsily G, Bidoglio G. A statistical method for source apportionment of riverine nitrogen loads. J Hydrol 2005;304:302–15.

Heathwaite AL, Johnes PJ, Peters NE. Trends in nutrients. Hydrol Process 1996;10: 263–93.

Helsel DR, Hirsch RM. Statistical methods in water resources. In: Chapter A3 B, Techniques of water-resources investigations of the United States geological survey, editor; 2002. p. 522.

Hirsch RM, Slack JR, Smith RA. Techniques of trend analysis for monthly water quality data. Water Resour Res 1982;18:107–21.

Hirsch RM, Alexander RB, Smith RA. Selection of methods for the detection and estimation of trends in water-quality. Water Resour Res 1991;27:803–13.

Howden NJK, Burt TP. Temporal and spatial analysis of nitrate concentrations from the Frome and Piddle catchments in Dorset (UK) for water years 1978 to 2007: evidence for nitrate breakthrough? Sci Total Environ 2008;407:507–26.

Ibáñez C, Prat N, Durán C, Pardos M, Munné A, Andreu R, et al. Changes in dissolved nutrients in the lower Ebro river: causes and consequences. Limnetica 2008;27:131–42.

Iital A, Stålnacke P, Deelstra J, Loigu E, Pihlak M. Effects of large-scale changes in emissions on nutrient concentrations in Estonian rivers in the Lake Peipsi drainage basin. J Hydrol 2005;304:261–73.

Järvet A, Mander Ü, Kull A, Kuusemets V. Nutrient runoff change in rural catchment in South Estonia. Archiv Hydrobiol 2002(141):305–19.

Kronvang B, Andersen HE, Børgesen C, Dalgaard T, Larsen SE, Bøgestrand J, et al. Effects of policy measures implemented in Denmark on nitrogen pollution of the aquatic environment. Environ Sci Policy 2008;11:144–52.

Lassaletta L. Flujos superficiales de nutrientes en una cuenca agrícola de Navarra. PhD thesis. Universidad Complutense de Madrid. Spain, 2007.

Libiseller C. A program for the computational of multivariate and partial Mann–Kendall tests. 2004. (http://www.mai.liu.se/~cllib/welcome/PMKtest.html).

Lorenz M, Nagel H-D, Granke O, Kraft P. Critical loads and their exceedances at intensive forest monitoring sites in Europe. Environ Poll 2008;155:426–35.

Merseburger GC, Martí E, Sabater F. Net changes in nutrient concentrations below a point source input in two streams draining catchments with contrasting land uses. Sci Total Environ 2005;347:217–29.

Meybeck M. Carbon, nitrogen, and phosphorus transport by world rivers. Am J Sci 1982;282:401–50.

Moreno JL, Navarro C, De las Heras J. Abiotic ecotypes in south-central Spanish rivers: reference conditions and pollution. Environ Poll 2006;143:388–96.

Négrel P, Roy S, Petelet-Giraud E, Millot R, Brenot A. Long-term fluxes of dissolved and suspended matter in the Ebro River Basin (Spain). J Hydrol 2007;342:249–60.

Oenema O, van Liere L, Schoumans O. Effects of lowering nitrogen and phosphorus surpluses in agriculture on the quality of groundwater and surface water in the Netherlands. J Hydrol 2005;304:289–301.

Oenema O, Oudendag D, Velthof GL. Nutrient losses from manure management in the European Union. Livest Sci 2007;112:261–672.

Oscoz J, Durán C, Pardos M, Gil J, Viamonte A. Historical evolution of the biological water quality in the Ebro Basin (Spain) (1990–2005). Limnetica 2008;27:119–30.

Oulehle F, McDowell WH, Aitkenhead-Peterson JA, Kram P, Hruska J, Navratil T, et al. Long-term trends in stream nitrate concentrations and losses across watersheds undergoing recovery from acidification in the Czech Republic. Ecosystems 2008;11: 410–25.

Peterson BJ, Wolheim WM, Muholland PJ, Webste JR, Meyer JL, Tank JL, et al. Control nitrogen export, from watersheds by headwater streams. Science 2001;292:86–90.

Pinilla V. The development of irrigated agriculture in twentieth-century Spain: a case study of the Ebro basin. Agric Hist Rev 2006;54:122–41.

Prat N, Munné A. Objetivos de estado ecológico en los ríos de la cuenca del Ebro. Zaragoza: Confederación Hidrográfica del Ebro; 1999. p. 58.

Radach G, Patsch J. Variability of continental riverine freshwater and nutrient inputs into the North Sea for the years 1977–2000 and its consequences for the assessment of eutrophication. Estuar Coast 2007;30:66–81.

Räike A, Pietiläinen OP, Rekolainen S, Kauppila P, Pitkänen H, Niemi J, et al. Trends of phosphorus, nitrogen and chlorophyll a concentrations in Finnish rivers and lakes in 1975–2000. Sci Total Environ 2003;310:47–59.

Rogora M. Synchronous trends in N-NO_3 export from N-saturated river catchments in relation to climate. Biogeochemistry 2007;86:251–68.

Sen PK. Estimates of the regression coefficient based on Kendall's tau. J Am Stat Assoc 1968;63:1379–89.

Sileika AS, Stalnacke P, Kutra S, Gaigalis K, Berankiene L. Temporal and spatial variation of nutrient levels in the Nemunas River (Lithuania and Belarus). Environ Monit Assess 2006;122:335–54.

Skjelkvåle BL, Stoddard JL, Jeffries DS, Tørseth K, Høgåsen T, Bowman J, et al. Regional scale evidence for improvements in surface water chemistry 1990–2001. Environ Poll 2005;137:165–76.

Skoulikidis NT, Amaxidis Y, Bertahas I, Laschou S, Gritzalis K. Analysis of factors driving stream water composition and synthesis of management tools—a case study on small/medium Greek catchments. Sci Total Environ 2006;362:205–41.

Stoddard JL. Long-term changes in watershed retention of nitrogen: its causes and aquatic consequences. In: Baker LA, editor. Environmental chemistry of lakes and reservoirs. Washington DC: Advances in Chemistry Series, 237.American Chemical Society; 1994. p. 223–84.

Tisseuil C, Wade AJ, Tudesque L. Modeling the stream water nitrate dynamics in a 60, 000-km2 European catchment, the garonne, Southwest France. J Environ Qual 2008;37:2155–69.

Torrecilla NJ, Galve JP, Zaera LG, Retamar JF, Álvarez ANA. Nutrient sources and dynamics in a Mediterranean fluvial regime (Ebro river, NE Spain) and their implications for water management. J Hydrol 2005;304:166–82.

Tudesque L, Gevrey M, Grenouillet G, Lek S. Long-term changes in water physicochemistry in the Adour-Garonne hydrographic network during the last three decades. Water Res 2008;42:732–42.

Turner RE, Rabalais NN, Justic D, Dortch Q. Global patterns of dissolved N, P and Si in large rivers. Biogeochemistry 2003;64:297–317.

Varela-Ortega C, Sumpsi JM. Repercusiones ambientales de la política agraria europea. In: Pineda FD, De Miguel JM, Casado MA, Montalvo J, editors. La diversidad biológica de España. S.A., Madrid: Pearson Educación; 2002. p. 125–52.

Ward MH, deKok TM, Levallois P, Brender J, Gulis G, Nolan BT, et al. Workgroup report: drinking-water nitrate and health-recent findings and research needs. Environ Health Persp 2005;113:1607–14.

Williamson CE, Dodds W, Kratz TK, Palmer MA. Lakes and streams as sentinels of environmental change in terrestrial and atmospheric processes. Front Ecol Environ 2008;6:247–54.

Yu Y-S, Zou S, Whittemore D. Non-parametric trend analysis of water quality data of rivers in Kansas. J Hydrol 1993;150:61–80.