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1 **Evolution Of N-balance With Qualitative Expert Evaluation Approach**

2

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8

10 **Abstract :** Pollution of rivers by nitrate is a major issue. Many land use units are considered as net
11 nitrate producers when the input dominates the uptake (e.g. agricultural areas), or in the opposite, net
12 consumers (e.g. wetlands), but the role of their spatial organization and temporal dynamics together
13 across the watershed is unclear. Here, we used a Nitrate-related Ecological Functions (NEF) concept,
14 together with an expert-based analysis in a Geographical Information System, to investigate the role of
15 two opposite landscape types in the nitrate regulation across the Garonne river watershed (France). At
16 any point in a watershed, there is nitrate production (NP) and nitrate removal (NR). The nitrate net
17 balance (NNB) between NP and NR functions can be neutral (NB, Neutral Balance) when nitrate
18 fluxes balance over space and time. The first landscape type, called *Actual*, was obtained using a set of
19 7 actual environmental variables, as land cover types, soil organic matter content and wetlands
20 presence. The second landscape type, called *Natural*, described a non-anthropized landscape, using the
21 same layer types as the *Actual* landscape. Potentials in NP and NR for each class in each map layer
22 were rated by a set of experts according to their scientific knowledge. NP, NR and by difference, NNB
23 maps were obtained, overlaid and compared to provide an evaluation of the potential for each
24 landscape. In both landscapes, NNB were largely balanced (*Actual* = 48% and *Natural* = 67%). In the
25 *Actual* landscape, NNB were secondly dominated by an imbalance toward NP (43%) and in the
26 *Natural* landscape secondly imbalanced toward NR (32%). We constructed ‘maps of disagreement’
27 between both landscapes to provide a spatially explicit assessment of NNB evolution caused by
28 changing land cover. We found that 67% of the agricultural areas and 60% of the artificial areas of the
29 watershed had been subjected to a loss of nitrate ecological functions from *Natural* to *Actual*
30 landscapes. Some management practices able to modify these factors may improve ecological
31 functions and diminish the NEF disagreement of the watershed.

32

33 **Keywords**

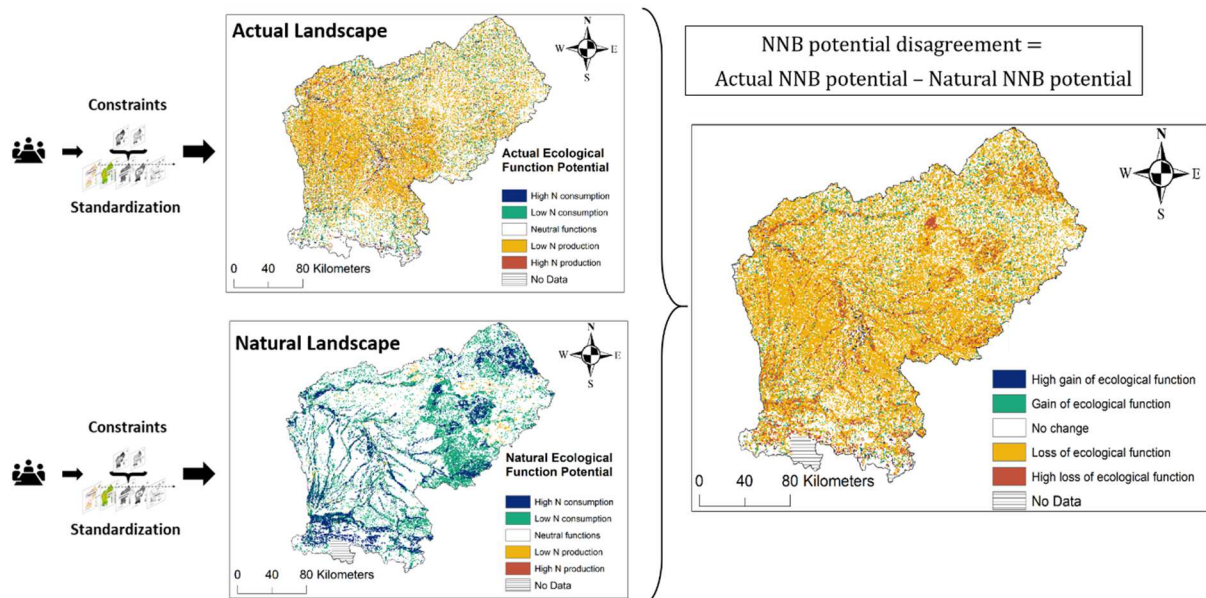
34 Expert-based analysis, landscapes, scenarios, nitrate removal, nitrate production, ecological functions

35 **Highlights**

- 36 • Scientific expertise could determine spatial trends of in-land nitrate production and removal
- 37 potential.
- 38 • Expert-based model allows to compare scenarios and to initiate debate between stakeholders.
- 39 • Agricultural and artificial areas are marked by loss of nitrate regulation.
- 40 • This study determines the level of human stressors on ecological functions.

41

42 **Graphical abstract (optional)**



43

44

45 1. Introduction

46 The use of land without caring for the environment has caused a loss of biodiversity and an
47 alteration of many ecological functions (Martín-López et al., 2018). Ecological functions gather the
48 physical, biological and biogeochemical processes occurring in an ecosystem (Goldstein, 2001;
49 Hooper et al., 2005; Jax, 2005; Martín-López et al., 2018).

50 Nitrate is a chemical compound which supports many ecological functions. Nitrate is a
51 nutrient leached from land to river, commonly used as a proxy of dissolved pollutants coming from
52 human stressors such as agriculture and cities (Cerro et al., 2014; Sánchez Pérez et al., 2003). Nitrate
53 is not severely toxic in itself but an excessive nitrate concentration (with excessive phosphorus) can
54 lead to eutrophic conditions in streams, which may degrade the water quality. Nitrate is introduced
55 into ecosystems by two main ways: (1) transformation of N compounds by chemical processes, or (2)
56 direct nitrate inputs. The chemical process of nitrification is the oxidation of ammonia into nitrate
57 thanks to ammonia-oxidizing microorganisms (Powlson, 1993). Nitrate inputs to the environment may
58 originate from natural (i.e., nitrate runoff, acid rain) and anthropogenic sources (organic and inorganic
59 fertilizers). In the following, we will consider that there is a nitrate production in the system through
60 either source.

61 Natural processes contribute to nitrate removal from soil and water column by denitrification
62 and N uptake by plants or algae, or others soil organisms. Denitrification is the reduction of nitrate
63 into N gas by denitrifying bacteria (Billen et al., 2013). Denitrification depends on the anaerobic
64 condition of the soil and the presence of soil organic carbon and nitrate. Nitrate naturally exists in soil
65 and water. In the environment and without any anthropogenic pressure, nitrate is either removed or
66 produced so that a sort of “balance” prevents environmental pollution. Too high nitrate inputs will
67 produce local pollution leading to ecosystem toxicity whereas the excessive development of plants
68 will lead to the eutrophication of the system, even if plants contribute to N uptake. Moreover, an
69 incomplete denitrification process will produce nitrous oxide, a potent greenhouse gas. The role of
70 land use is important in the N reactions. The risk of imbalance in the nitrate-related processes is high in
71 urban and intensive agricultural areas whereas lands occupied by forest and permanent grasslands
72 present a smaller risk. Most croplands will diminish the nitrogen soil compound except for leguminous
73 crops, whereas some land uses as grasslands and pastures will promote N processes. Wetlands, in
74 particular, are well-known to have a high N processes activity (Fisher and Acreman, 2004; Hansen et
75 al., 2018; Martínez-Espinosa et al., 2021; Sánchez-Pérez et al., 2003).”

76 Although N cycling in ecosystems is well known, the quantification of N processes through
77 different pathways at the watershed scale remains challenging (Grizzetti et al., 2015). Few models
78 such as STOMOD (Reiche et al., 1996) and SWAT (Arnold et al, 2012) were developed to quantify N
79 fluxes and balances in the watershed. These models quantify the real N balance at large scale including
80 soil capacity as a sink of N that can be lower than the potential one. It is challenging to quantify the

81 net balance of all processes. Nitrate Net Balance (NNB) represents the net balance of all nitrate-related
82 processes. The NNB can be divided into three categories: nitrate removal (NR), nitrate production
83 (NP) and neutral balance (NB). Studies that worked on the NR and NP processes are either field-based
84 (Burt et al., 1999), based only on stream processes (Sauvage et al., 2018), or integrated into complex
85 dynamic modeling (Cakir et al., 2020). Some static studies of the nitrate balance were also conducted
86 but either focused on the nitrate mass balance at the outlet of the watershed (Sutton et al., 2011) or
87 targeted only one kind of process (Burt et al., 1999; Revsbech and Sørensen, 1990).

88 In this study, we aimed at quantifying the landscape NNB potential based on a mapping
89 methodology and expert knowledge to highlight the possible alteration of NNB that would result from
90 a changing landscape. The study takes place from the water stakeholder point of view focusing on the
91 water purification service. This mapping should highlight hot spots which are the places where the
92 NNB is the most active according to Vidon et al. (2010) and the areas where human activities have an
93 impact on nitrate-related ecological functions. The methodology is based on a multi-criteria analysis
94 comparing the actual landscape, called *Actual landscape*, and a potential one without any human
95 stressors, called *Natural landscape*.

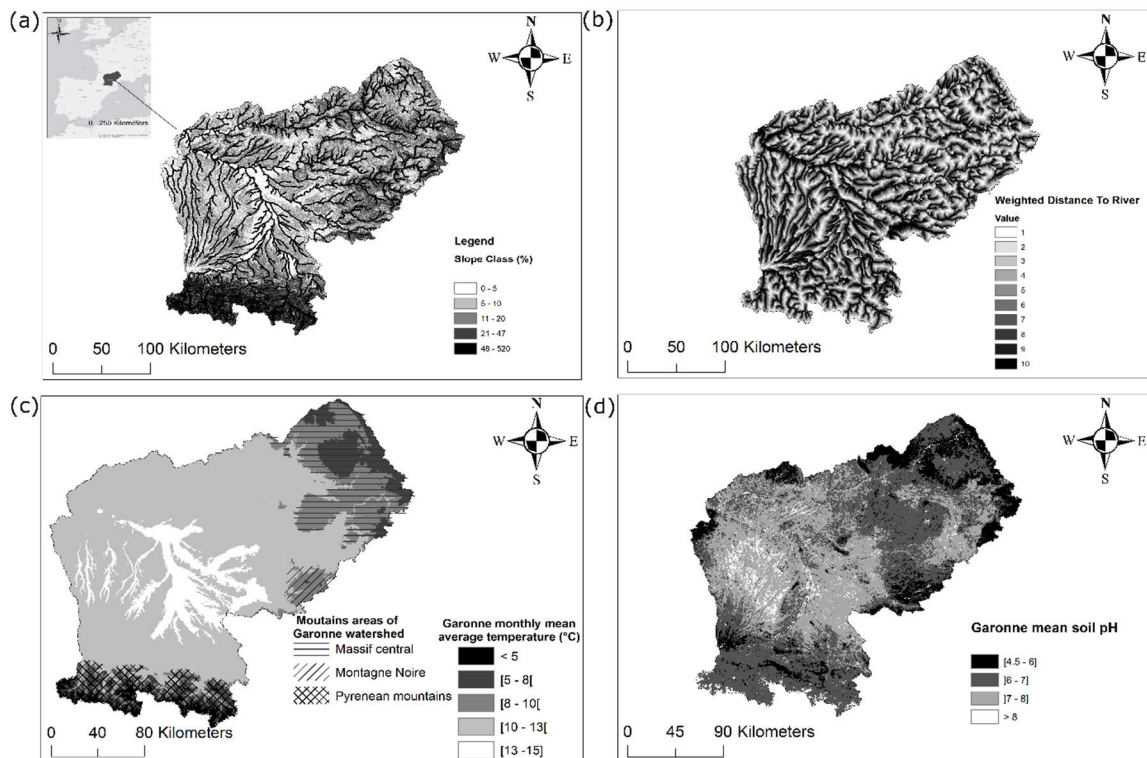
96 2. Material and methods

97 2.1. Study area

98 The study area encompasses the hydrographic basin of the Garonne River, an area of
99 approximately 50,000 km² located in the South of France with Toulouse and Bordeaux as the main
100 cities (Figure 1a). This methodology has been tested on the Garonne basin where a lot of information
101 is available and where several studies were conducted (Biancamaria et al., 2019; Cakir et al., 2020;
102 Espitalier-Noël et al., 2016). The area is very diverse in terms of pedoclimatic conditions, with three
103 specific geographic entities (Figure 1c): the plain downstream of the basin, the Pyrenean Mountains in
104 the South and the Massif Central in the North-East (Tockner et al., 2009). The watershed is influenced
105 by three climatic zones, with Mediterranean climate conditions in the East, a mountain-continental
106 type in the South and an altered oceanic climate in the North and West part (Joly et al., 2010; Tockner
107 et al., 2009). In the central part of the watershed, soils are mainly developed from calcareous materials
108 leading to high soil pH (Figure 1d). On the crystalline basement of the western and southern borders,
109 more acidic soils can be found. The most representative soils are Cambisols (54.4% of the basin area),
110 Leptosols (14.7%), Fluvisols (6.8%) and Luvisols (Revel and Guiresse, 1995). Cambisols make good
111 agricultural lands and are intensively used. Leptosols are very shallow soils. Fluvisols are located in
112 the floodplains. They are of great economic value for agriculture. Since they are connected to the
113 groundwater, nitrate losses can occur. Some Luvisols can also be found in the oldest fluvial terraces.
114 The Garonne watershed is subjected to intense human activities (Dynesius and Nilsson, 1994) such as
115 agriculture (60% of the watershed area), water management systems (daily discharges are influenced
116 by 210 dams) and urban impact by 5 million inhabitants. The agricultural area is characterized by low-

117 slopes and soils easy to till. In the floodplain, land use is dominated by annual cropping systems and
 118 urban areas whereas the hillsides are occupied by pastures, pines, mixed forests and shrublands
 119 (Pautou et al., 2003). Wetlands cover 232 km² equivalent to 0.45% of the Garonne basin area.

120 Without any human influence, the watershed might be covered entirely by forests, including
 121 45% of various oak forests according to the natural potential vegetation map (Legu dois et al., 2011).
 122 As a result, the soil organic matter content in the pristine conditions of the Garonne watershed is
 123 higher than in the Actual soil. Finally, in the pristine version of the watershed, wetland areas are more
 124 developed. Indeed, the world has lost at least 50% of its wetlands (Davidson, 2014).

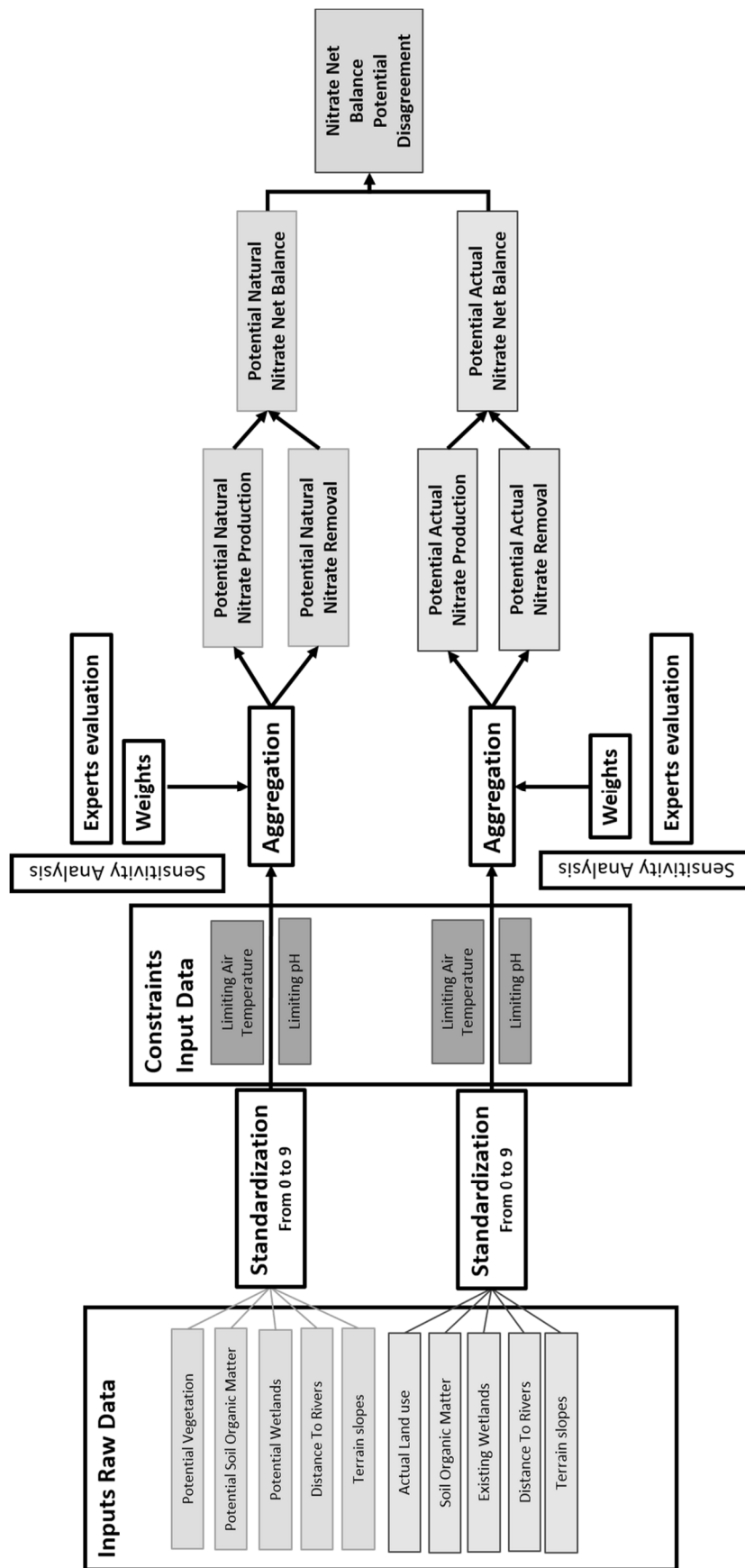


125
 126 *Figure 1: (a) Localization of the Garonne basin, slope map and main streams (~90m; NASA, 2011). (b) Weighted distances*
 127 *to the river classified in ten percentile ranks (~90m; NASA, 2011). (c) Average monthly mean temperature and localization of*
 128 *mountains area in the Garonne watershed (~1km; Fick and Hijmans, 2017). (d) Average soil pH (1/250,000; Guiresse et*
 129 *al., 2019; Bornand et al.,1995).*

130 2.2. Predicted variables (NP & NR)

131 NNB potential was investigated using an expert-based spatial multi-criteria analysis in a
 132 Geographical Information System (Figure 2). The multi-criteria analysis is known as a spatial decision
 133 support system (Malczewski, 2006) and is used in several studies (Garfi et al., 2011; Junior et al.,
 134 2014; Macary et al., 2013; Pacheco and Sanches Fernandes, 2016). The spatial multi-criteria analysis
 135 was applied to two landscapes: (1) an *Actual landscape*, using datasets characterizing the present
 136 conditions of the watershed and (2) a *Natural landscape*, representing an idealized non-anthropized
 137 landscape close to its pristine state. The overlay of the output results of both landscapes led to a
 138 disagreement map which indicated a loss or a gain of ecological function potential under human
 139 stressors.

140 The NNB potential is the net balance between NR and NP functions. The predicted variables
141 are NR and NP. Functions definitions are crucial to select adequate input datasets.



142

143 *Figure 2: Flowchart illustrating the general methodology of the multi-criteria model used to assess the Nitrate Net Balance*
 144 *(NNB) disagreement map.*

2.3. Predictive variables

Data obtained from several providers were used for the prediction of NP and NR. Some data were used for the *Actual landscape* model and/or the *Natural landscape* model as detailed in Table 1.

The land cover was considered for its effect on NR and NP potentials (Cakir et al., 2020; Colombo et al., 2015; Groffman et al., 2004; Groffman and Tiedje, 1989; Migliaccio et al., 2007; Vidon et al., 2010). Land cover maps usually do not represent wetlands whereas wetlands are hot spots of nitrogen cycle processes (Anbumozhi et al., 2005; Brinson and Malvárez, 2002; Fisher and Acreman, 2004; Hansen et al., 2018; Hefting et al., 2013; Maltby, 2009; Montreuil et al., 2010). Therefore, we used a specific map delineating the presence of wetlands (Adour-Garonne Water Agency et al., 2011, Table 1). The soil organic matter was also integrated into the model as a proxy of the nitrification and denitrification processes given that soil organic matter is one reagent of these processes (Dodla et al., 2008; Khademikia et al., 2013; Sebestyen et al., 2014). The distance to the river was examined as a proxy of soil humidity on which nitrification, mineralization and denitrification depend (Hefting et al., 2005; Macary et al., 2013). The terrain slope was used to modulate the nitrate loss from soil and the processes kinetics (Cakir et al., 2020; Caubel, 2001; Macary et al., 2013; Revel and Guiesse, 1995). High slopes favor nitrate loss through subsurface lateral flow and limit nitrate-related processes because of fast water transit time in soil. Finally, we considered two additional datasets limiting the nitrate-related processes. Firstly, air temperature was used as a proxy of soil temperature (Stefan and Preud'homme, 1993). Soil temperature can be simulated with air temperature based on regression analysis (Barman et al., 2017; Zheng et al., 1993). We decided to consider soil temperature boundaries to define air temperature ones as most of the regressions have a slope near to 1. Soil temperature under 5°C or above 40°C limits nitrogen cycle processes whereas the optimum temperature is around 25°C (Piñeiro et al., 2010; Revel and Guiesse, 1995; Ullah et al., 2005; Willems et al., 1997). Secondly, soil pH values inhibit, restrict, or activate nitrogen cycle processes (Abalos et al., 2019; Stevens et al., 1998). Very acidic soils (pH lower than 4) will inhibit processes whereas processes are activated for pH between 7 and 8.6.

Distinct datasets of soil organic matter content, land cover and delineation of wetlands were used to generate the *Actual* and *Natural conditions*. These datasets were considered to be highly influenced by human stressors. On the contrary, soil pH, distance to the rivers and topographic data were used to build both *Actual* and *Natural conditions* maps. This study assumed that there is no difference in the air temperature and topography between the *Actual* and *Natural landscapes* (Figure 2).

In the *Actual landscape* model, we used a land cover gridded map of the year 2017. This map has been produced by the French Center for the Study of the Biosphere from Space (CESBIO) and is freely accessible at <http://osr-cesbio.ups-tlse.fr/~oso/> (Derksen et al., 2020). The pixel has size at ground of approximately 10 m and is classified into 17 land use classes (Figure 3a). In the *Natural*

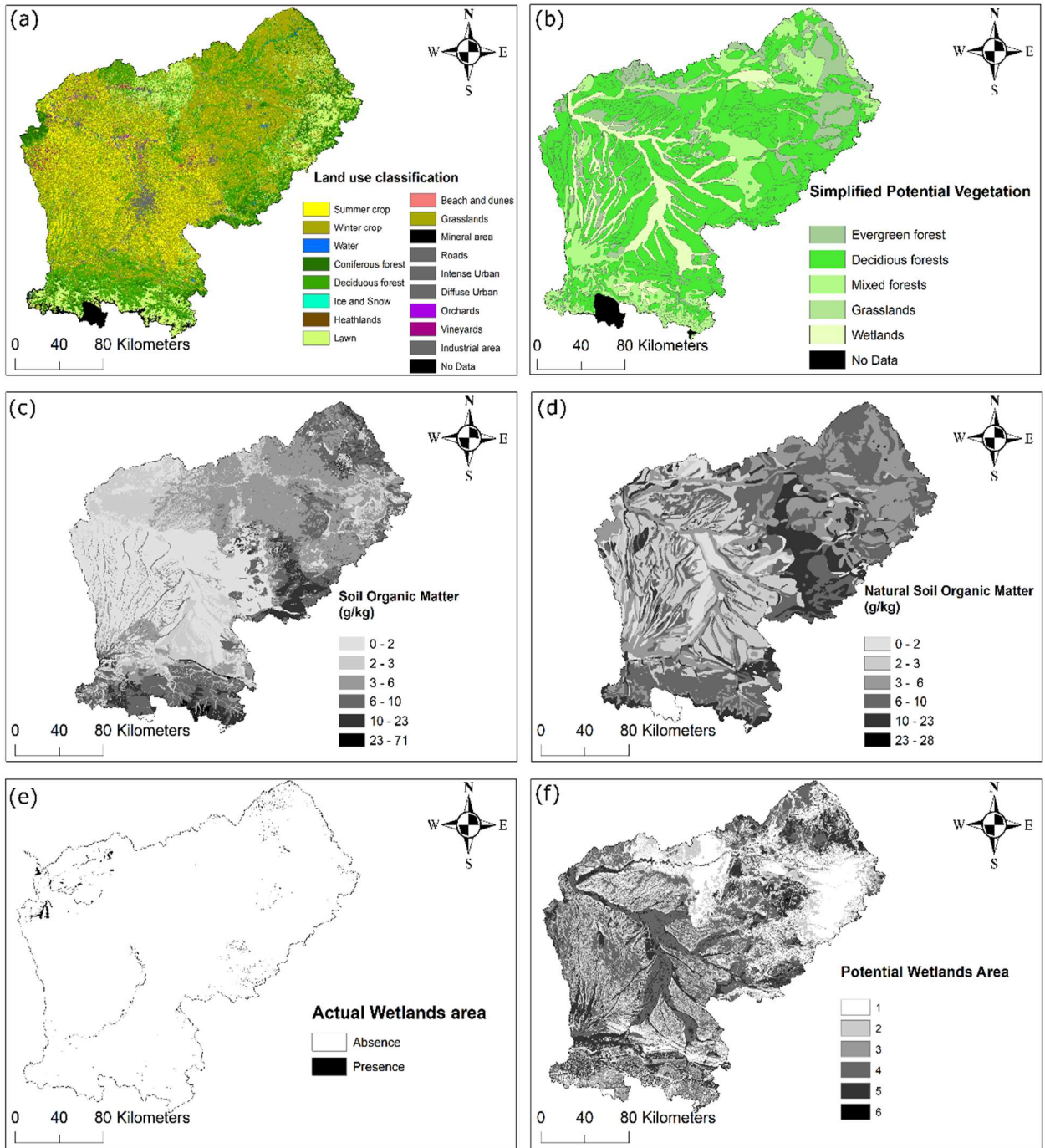
181 *landscape*, we used the geographical database of the « Carte de la Végétation Potentielle de la
182 France » at a scale of 1/1 250,000 ([https://cdn.obs-mip.fr/cartevegetation/Carre-vegetation-](https://cdn.obs-mip.fr/cartevegetation/Carre-vegetation-potentielle.html)
183 [potentielle.html](https://cdn.obs-mip.fr/cartevegetation/Carre-vegetation-potentielle.html)), which is a collaborative work of the French National Centre for Scientific Research
184 (CNRS), Paul Sabatier University (UPS), National Polytechnique Institute of Toulouse (INPT),
185 National Institute of Agronomic Research (INRA), University of Nancy I, University of Aix Marseille,
186 AgroParistech-ENGREF (declaration d'invention « Base de données » DI 00461-03) (Leguédou et al.,
187 2011). Potential vegetation describes the vegetation "that can be expected to recover given the
188 partially irreversible changes to the environment" (Ozenda and Lucas, 1987; Figure 3b). The potential
189 vegetation in the Garonne basin is mainly composed of deciduous forests (58%), mixed forests (19%),
190 wetlands (12%) and evergreen forests (11%). Since we considered the map of potential vegetation the
191 *Natural landscape* depicted will be an exaggeration of the pristine state since the landscape will not
192 endure human and environmental stressors such as arsons or natural fires.

193 Vector maps of soil organic matter content (Figure 3c), as well as soil pH (Figure 1d), came
194 from the "Référentiel Régional Pédologique" database at 1:250,000 scale, mapped by several regional
195 producers (Table1). The average soil organic matter contents and average soil pH values were
196 calculated within the first 30 cm of soil. For the *Actual landscape* model, the soil maps did not cover
197 the entire Garonne basin and an extrapolation of the actual soil-related properties was achieved in the
198 missing area as explained in Appendix A. For the *Natural landscape* model, the potential map of soil
199 organic matter content was set from the *Actual* maps and field-observations as described in Appendix
200 A (Figure 3d). Wetland vector maps were produced by the French Water Agency in collaboration with
201 the Forum des Marais Atlantiques (FMA) at the scale 1:50,000. In the *Actual landscape* model, we
202 used a map obtained by field inventories and analysis of remote sensing data (Figure 3e) whereas, in
203 the *Natural landscape* model, a potential wetland map based on the basin and river network
204 morphology was considered (Figure 3f). Wetlands field inventories respect the wetlands definition
205 provided by the French legislation (Article L211-1, 2006; Article R211-108, 2006) which does not
206 take into account all wetland areas as defined by the scientific community. More details on the
207 potential wetlands map are provided in Adour-Garonne Water Agency et al. (2011). For both
208 landscape models, the Global Digital Elevation Model V2 was used to calculate the slope (Figure 1a)
209 and altitude-weighted distance to rivers (Figure 1b). Data were acquired by the governmental U.S.
210 agency, National Aeronautics and Space Administration (NASA, 2011) with a ground pixel size of
211 approximately 90 m. This scale, used in previous studies (Cakir et al., 2020 . Biancamaria et al.,
212 2019), is sufficient to evaluate the distance to the river and slopes to distinguish alluvial plains and
213 wide valleys. In this study, slope variable is fixed since at the scale of the basin area, we considered
214 that there is no change between *Natural* and *Actual Landscapes*. Monthly average of air temperature at
215 ground level from the WorldClim version 2.1 climate data for 1970-2000 were downloaded at
216 <https://www.worldclim.org/data/index.html>. This version was released in January 2020. The data is

217 available at the 30 seconds ($\sim 1 \text{ km}^2$) spatial resolution. It consisted in 12 layers, one for each month.
218 The annual average of air temperature at ground level was calculated thanks to Arcgis tools and was
219 used in this study for both landscapes (Figure 1c).

Table 1: Datasets used in this study to determined potential maps of both Actual and Natural landscapes.

	Data	Purpose	Owner institution	Source
Actual landscape	Land use map ~10 m (OSO)	Actual land use is used for its effect on the nitrate production (NP) and nitrate removal (NR) potentials	CESBIO, France	http://osr-cesbio.ups-tlse.fr/~oso/
	Soil organic matter content within the first 30 cm of soil (Référentiel Régional Pédologique)	Assessment of one of the reagents of the nitrification and denitrification reactions	CNRS, France INRAE, France	Guireesse et al. (2019), Bornand et al. (1995) https://www4.obs-mip.fr/rp-midi-pyrenees/ https://www.umr-lisah.fr/
	Actual wetlands of the Adour-Garonne basins	Identification of the wetlands in the watershed where nitrogen cycle processes are more active	French Water Agencies, France	Adour-Garonne Water Agency et al. (2011)
Natural landscape	Potential vegetation of France	Assessment of the theoretical land use without human stressors	DI 00461-03	Leguédois et al. (2011), Party et al. (2015) - https://cdn.obs-mip.fr/cartevegetation/Carre-vegetation-potentielle.html
	Soil organic matter content within the first 30 cm of soil, modified according to the potential vegetation (Référentiel Régional Pédologique)	Assessment of one of the reagents of the nitrification and denitrification reactions	CNRS, France INRAE, France	Guireesse et al. (2019), Bornand et al. (1995) https://www4.obs-mip.fr/rp-midi-pyrenees/ https://www.umr-lisah.fr/
	Potential wetlands of the Adour-Garonne Basins	Assessment of the probability of having wetlands in the watershed according to morphology and river network data	French Water Agencies, France	Adour-Garonne Water Agency et al. (2011) https://www.data.gouv.fr
Data common to both landscapes	Global Digital Elevation Model V2 ~90m	Calculation of the distance to the river and slope, as proxies of the nitrate loss and soil humidity	NASA, USA	NASA (2011)
	Air temperature grid ~1 km (WorldClim)	Air temperature is used as a proxy of soil temperature which limit or activate nitrogen cycle processes	Feed The Future, USA	Fick and Hijmans (2017) https://www.feedthefuture.gov/
	Soil pH 1/250,000 (Référentiel Régional Pédologique)	Soil pH can inhibit, restrict or activate nitrogen cycle processes	CNRS, France INRAE, Lisah	Guireesse et al. (2019), Bornand et al. (1995), https://www4.obs-mip.fr/rp-midi-pyrenees/ , https://www.umr-lisah.fr/



221

222 *Figure 3: Input data for multi-criteria analysis. (a) Actual Land use map ; (b) Simplified Potential Vegetation under Natural*
 223 *landscape ; (c) Soil organic matter content in g/kg for Actual landscape; (d) Soil organic matter content in g/kg for Natural*
 224 *landscape; (e) Actual wetlands ; (f) Potential Wetland Areas (1: low probability of wetland presence – 6: high probability of*
 225 *wetland presence).*

2.4. GIS analysis workflow for mapping NNB potential

All predictive variables (Figure 1 and Figure 3) were converted into gridded layers when needed and values were standardized by reclassification to a common measurement scale of 10 classes of NP potential and 10 other classes of NR potential (Figure 2). The gridded layers had a pixel size of 1 km. Air temperature and pH grids were only used as limiting input data. Values of soil organic matter content, distance to the river and slopes were reclassified into 10 classes of equal size for the *Actual landscape* maps based on ten percentile ranks. The same class thresholds were used for the *Natural* datasets to have equity between both *Actual* and *Natural landscapes* in the attribution of potentials. This equity is important to compare the model outputs of these two landscapes. A group of 10 scientific experts in biogeochemistry were asked, based on their expertise, to rate each class of each predictive variable from 0 to 9, regarding their potential in NP and NR, i.e. 0 meaning “no process occurs” and 9 “the maximal process occurs”. Precisely, the experts were asked to assign a score for the NP and NR potentials for each attribute of each variable, and those for each of the scenarios. The expert scorings were validated based on the knowledge of the study site and different studies that have been done (Fabre et al., 2020; Ranalli and Macalady, 2010; Sánchez Pérez et al., 2003; Sánchez-Pérez et al., 2003; Yao et al., 2017). The work was carried out in three stages: (1) the first stage where the experts were asked to list the available databases that could be used to assess NR and NP potentials, (2) the second stage where each expert was asked to design the scoring rules according to their expertise and (3) the third stage of consultation where the results were collated. In general, the experts assigned the same scores and in case of discrepancies, a debate took place to reach a consensus. The experts considered that the selected variables are enough to evaluate N-balance over the entire watershed. Layer combinations will help to distinguish different landscapes. For example, narrow valleys will be defined by short to medium distances to the river and high slopes whereas wide valleys will be defined by wetlands presence, high slope and high distance to river. The results of this work are presented in Table 2. Each reclassified variable was assigned an equal weight of 20%. The assignation of weights can be done by expert decision or defined thanks to statistics. In this study, the weighting of input data with an equal weight was determined using the Analytic Hierarchy Process (AHP) developed by Saaty (1990) and used in many studies (Siddiqui et al., 1996; Junior et al., 2014; Vaidya and Kumar, 2006). More details are provided in Appendix B. The scores of each input data were multiplied by their weight and added together. Finally, temperature and pH were integrated in the equation as limiting factors with a multiplication by their score (Eq. 1).

$$P = \sum_{i=0}^n 0.2 * X_i * \prod_{j=1}^k Y_j \quad (\text{Eq. 1})$$

X_i is the score of the input data i varying from 0 to 9, Y_j is the score of the limiting input data j , n is the number of input data (= 5) and k is the number of limiting input data (= 2). In the end, P values were classified into ten potential classes from very low potential to very high potential.

260 Thanks to the combination of all predictive variables, this methodology permitted to produce two
261 maps for each landscape, with a pixel size of 1 km: a map of the NP potential and a map of the NR
262 potential. The difference between NP and NR potentials corresponds to the NNB potential (Eq. 2) as the
263 combination of all the potentials (NP and NR) occurring in the Garonne basin. A positive value means that
264 nitrate is produced in the area whereas a negative value means that the area removes nitrate. A potential
265 equal to zero indicates that the balance is neutral (Nitrate Balance, NB). The NNB potential map is
266 generated for both landscapes: the *Actual* and the *Natural* ones.

$$267 \qquad \qquad \qquad \text{NNB potential} = \text{NP potential} - \text{NR potential} \qquad \qquad \qquad (\text{Eq. 2})$$

268 To validate the model, we used previous studies performed in the Garonne basin and compared our
269 NR potential with the annual rates of soil microbiological processes. Denitrification rate is chosen because
270 it is one of the most important NR processes with plant uptake. The first database resulted from the study
271 of Pinay et al. (1995). The samplings (52 in total) were taken each month from October 1990 to November
272 1991 in two riparian sites of the Garonne River downstream of Toulouse (Verdun-sur-Garonne and
273 Grenade sites). The second database was collected during the ATTENAGUA project from April 2013 to
274 March 2014. 4 campaigns were achieved under different flow landscapes in the riparian zone of 50
275 ha close to the village of Monbequi (Yao et al., 2017). Inside the riparian zone, three types of land use
276 were sampled: the agricultural area, the riparian forest area and the intermediate area.

277

278 *Table 2: Scoring rules designed by scientific experts in biogeochemistry for each predictive variable regarding nitrate production*
 279 *(NP) and nitrate removal (NR) potential for both Actual and Natural landscapes. The score is ranging from 0 to 9, 0 meaning “no*
 280 *process occurs” and 9 “the maximal process occurs”.*

Condition	Input data	Scoring rule	Illustrative diagram	Explanation
Actual	Land use	Attribution of discrete potential score		Forests produce and remove nitrate and so have a net-zero balance of nitrate. Agriculture has a positive balance of nitrate because of the fertilizer applications. Urban area releases and eliminate nitrates thanks to waste water treatment plant, the balance is positive.
	Wetlands	Attribution of discrete potential score: 9 if presence of wetland, 0 if not		Wetlands are nitrate producer and consumer, the balance is negative (more nitrate is eliminated). In no-wetlands area, a same potential of nitrate production is applied.
	Soil Organic Carbon	Increasing sigmoid function		Soil with high organic carbon content activated nitrate cycle processes including nitrate production and removal. The potential trends are similar for both processes and the net balance is equal to zero. For a certain amount of organic carbon, there is a platter effect.
Natural	Land use	Attribution of discrete potential score		Forests produce and remove nitrate and so have a net-zero balance of nitrate. In this scenarios, there is no difference in nitrate production-removal potential
	Wetlands	Reclassification of the potential provided in the datasets		The potential of existing wetlands varying from 1 to 6. The class 5 and 6 correspond to wetland presence. Wetlands are nitrate producer and consumer, the balance is negative (more nitrate is removed). In no-wetlands area, a same potential of nitrate production and nitrate removal is applied.
	Soil Organic Carbon	Increasing sigmoid function		In natural scenarios, the soil organic carbon content will have an effect on the nitrogen cycle processes. Spot with low organic carbon content will not permit the nitrogen cycle reaction to reach the denitrification whereas nitrate production reactions will first occur in a spots with low organic carbon content.
Common to Actual and Natural	Distance to water courses	Decreasing or Increasing sigmoid		The distance to river is a proxy soil humidity and also a proxy of potential leaching. The nitrate production potential of an area is increasing with river distance with aerobic soil and ripisylve absence. In the opposite, the nitrate removal potential is higher closest to the river with hypothetically a presence of ripisylve and anoxic soil.
	Terrain slope	Increasing sigmoid function		Smooth slopes promote nitrate cycle processes whereas steep slopes avoided processes and encourage leaching.
Common to Actual and Natural	Limiting input data	Scoring rule	Illustrative diagram	Explanation
	pH value	Levels of inhibition and activation of processes		Nitrogen cycle processes are optimal when pH is between 7 and 8.6. In this case the potential of 10 is set up. The N processes are partially inhibited for a pH above 8.6 or between 4 and 7, in this case potential of 5 is set up. N processes are completely inhibited for a pH lower than 4 (potential = 0).
Air temperature	Levels of inhibition and activation of processes		Nitrogen cycle processes are inhibited for a temperature lower than 5°C and above 40°C. Nitrate production potential is activated at the maximum potential between 23 and 30°C. Nitrate removal potential is maximal between 23°C and 40°C. A potential of 5 had been arbitrary set up for the rest intervals to represent spots where reactions can occur without any temperature level activation.	

2.5. Mapping Disagreement Between *Actual* And *Natural* Landscapes

Maps of NP and NR potentials were overlaid to obtain a map of disagreement between both landscapes in order to detect locations of potential loss of NR and NP potentials between both landscapes due to anthropization. An analysis of NNB functions (NP, NR and NB) partitioning by zones had been led. Zones were built from an overlay of land cover types of both *Actual* and *Natural* landscapes according to Table 3.

The NNB potential disagreement map is the deviation of the *Actual* NNB map from the *Natural* NNB map:

$$\text{NNB potential disagreement} = \text{Actual/NNB potential} - \text{Natural/NNB potential} \quad (\text{Eq. 3})$$

According to water quality management, a positive NNB increases the risk of water quality degradation; on the contrary, a negative NNB indicates an improvement in water quality. Therefore, our hypothesis states that a positive NNB disagreement indicates a loss of Nitrate-related Ecological Functions (NEF) in the *Actual landscape* as compared to *Natural landscape*. Three possible cases are giving a positive NNB disagreement: (1) an increase of NP from *Natural* to *Actual* landscapes, (2) a decrease of NR from *Natural* to *Actual* landscapes, or (3) a loss of function, i.e. an area characterized by NP potential ($\text{NNB} > 0$) in *Actual landscape* and by a NR or NB potential ($\text{NNB} \leq 0$) in *Natural landscape*. In the same way, a negative value demonstrates a gain of NEF from *Natural* to *Actual landscape* and a neutral value indicates no change in NNB potential, meaning that there is no difference of NEF in the area between both landscapes. The disagreement is graduated from -9 to +9.

Usually, the water management decision plan is established at the upper-scale level, such as the entire territory level or the level of the water bodies, called subbasins in this study. We proceeded to an aggregation of the NNB maps for both landscapes and the disagreement map at the level of the subbasins. This study distinguished 3 classes for NNB potential: NB, NR and NP. A classification of these three classes has been done from the most to the less frequent occurrences in each subbasin. The same classification was made with the NNB disagreement maps with the following classes: no change, gain of NEF and loss of NEF. In the subbasin x , the percentage of surface area occupied by the NNB potential class i is generated from the number of pixels of the class i of the subbasin x , divided by the total number of pixels of the subbasin x . We also calculated the subbasin potential of each class in each subbasin with the following equation:

$$\text{Subbasin potential}_x = \frac{\sum_{i=1}^n \text{Potential}_i \times \text{Number of pixels}_i}{\text{Number of pixels}_x} \quad (\text{Eq. 4})$$

With x the subbasin number, n the number of potential class considered, Potential_i the potential value i varying from - 9 to + 9, $\text{Number of pixels}_i$ of the potential i in the subbasin x . The calculation of the subbasin potential was done for the three classes: NP ($\text{NNB} > 0$), NR ($\text{NNB} < 0$) and NB ($\text{NNB} = 0$). The

315 same work had been led on NNB disagreement potentials. For the gain of NEF, the NNB disagreement
 316 potential value was considered to vary from -9 to -1. For the loss of NEF, we considered the NNB
 317 disagreement potential value varying from +1 to +9. Finally, for the no-change class equal to 0, we only
 318 considered the number of pixels in each subbasin ($Potential_i=0$).

319 *Table 3: Zonal repartition considering land cover maps of Actual and Natural landscapes used in results analysis.*

Zone	Zone	Description <i>Actual</i>	Description <i>Natural</i>	Percentage relative to the total area	Percentage relative to each <i>Actual</i> land use zone
ZA*	ZA_1	Agricultural areas	Deciduous forests	37.5%	63%
	ZA_2	Agricultural areas	Evergreen forest	5.4%	9%
	ZA_3	Agricultural areas	Grasslands	0.0%	0%
	ZA_4	Agricultural areas	Mixed forest	8.3%	14%
	ZA_5	Agricultural areas	Wetlands	8.7%	15%
ZU*	ZU_1	Artificial surfaces	Deciduous forests	1.2%	45%
	ZU_2	Artificial surfaces	Evergreen forest	0.1%	4%
	ZU_3	Artificial surfaces	Mixed forest	0.3%	10%
	ZU_4	Artificial surfaces	Wetlands	1.1%	41%
ZF*	ZF_1	Forest and semi natural areas	Deciduous forests	18.4%	52%
	ZF_2	Forest and semi natural areas	Evergreen forest	5.7%	16%
	ZF_3	Forest and semi natural areas	Grasslands	0.0%	0%
	ZF_4	Forest and semi natural areas	Mixed forest	9.6%	27%
	ZF_5	Forest and semi natural areas	Wetlands	1.5%	4%
ZWa*	ZWa_1	Water bodies	Deciduous forests	0.2%	52%
	ZWa_2	Water bodies	Evergreen forest	0.0%	5%
	ZWa_3	Water bodies	Mixed forest	0.1%	39%
	ZWa_4	Water bodies	Wetlands	1.6%	4%
ZWe*	ZWe_1	Wetlands	Deciduous forests	0.0%	2%
	ZWe_2	Wetlands	Wetlands	0.1%	98%

320 *ZA : Agricultural zone ; ZU : Urban zone, ZF : Forests zone, ZWa : Water Bodies zone, ZWe : Wetlands zone.

321 3. Results

322 3.1. NNB potential maps

323 Concerning NP potential for both landscapes, the production of nitrate (Figures 4-1a and 4-2a) is
 324 limited by soil temperature, especially in the high altitudes of the Pyrenean mountains. For both
 325 landscapes, the NP potential is higher in floodplains and near rivers and the NP potential decreases in the
 326 upland parts of the watershed because of the topography. The frequency distribution of NP potentials for
 327 both landscapes follows a standard normal pattern. The most frequent NP potential for the *Actual*
 328 *landscape* is class +4 with 38.1% of the basin area (Figure 4-1a), whereas class +3 is the most frequent
 329 one in the *Natural landscape* with 64.9% of the basin area (Figure 4-2a). 43.3% of the basin area shows

330 the same NP potential in both landscapes, whereas 42.5% of the Garonne area has a higher NP potential in
331 the *Actual landscape*, meaning that there is a gain of NP potential going from *Natural* to *Actual*
332 *landscapes*.

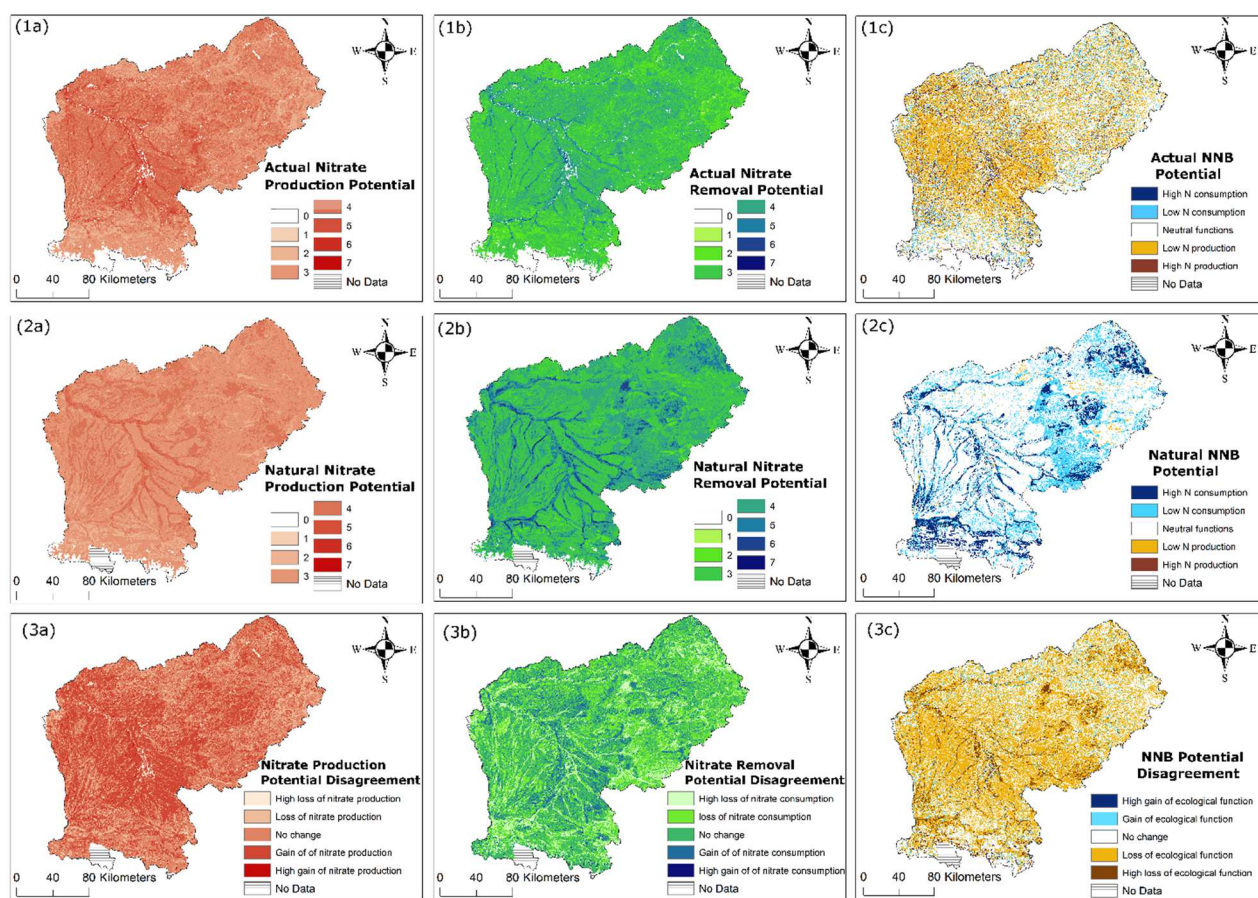
333 The NR potential is also restricted by soil temperature in the Pyrenees and cities. As for NP
334 potential, the floodplains and the river systems are characterized by a high NR potential for both
335 landscapes (Figures 4-1b and 4-2b). NR potential values for both landscapes follow a standard normal
336 pattern with a highest occurrence of NR potential of +3 representing 57.5% and 46.5% of the basin area
337 for *Actual* and *Natural landscapes*, respectively. For inlands, near the river, under *Natural landscape*, NR
338 potential is higher than the NR potential in the *Actual landscape*: there is a loss of NR going from *Natural*
339 to *Actual landscapes*. The North-East and the East part of the watershed in the Massif Central mountain
340 and the Montagne Noire are characterized by high values of NR in the *Natural landscape*. Figure 4-3b
341 shows the disagreement in NR potential between the *Actual* and *Natural landscapes*. This figure shows
342 that 46.3% of the basin area consumes more nitrate in the *Natural landscape* whereas there is no change
343 for 42.4% of the basin area between both landscapes and 11.4% of the basin area consumes more nitrate in
344 the *Actual landscape* (Figure 4-3b). In this latter case, when the NR potential is higher in the *Actual*
345 *landscape* (gain of NEF), the NR potential difference is usually low, with a difference of +1 for 90% of
346 the area under consideration. These areas are characterized by a high distance to the river, high slope, low
347 wetlands potential and low soil organic matter content. According to Figure 4-3b, the gain of NR happens
348 mostly in the alluvial part of the basin.

349 The difference between NP and NR potentials gives the potential of the Nitrate Net Balance
350 (NNB) (Figure 4-1c for *Actual landscape* and Figure 4-2c for *Natural landscape*). NNB values follow a
351 standard normal distribution for both landscapes.

352 For the *Natural landscape*, NB, the balance between NP and NR, showed that more than half of
353 the watershed exhibits an equilibrium in regulating nitrate. Indeed, 66.9% of the area is rated as $NNB = 0$
354 whereas only 1.1% of the basin area is characterized by NP. Moreover, 87% of the *Natural* $NP = +1$,
355 which is a low NP potential (NP varying between +9 and +1). The remaining area (32.1%) has a negative
356 NNB meaning a NR activated by the self-purification service of the landscape and 83% of this negative
357 NNB has a potential equal to -1. The *Natural* negative NNB (Figure 4-2c), indicating a NR, is located
358 mainly along rivers (short distance to the river), wetlands and mountains. For the *Actual landscape* (Figure
359 4-1c), 48.5% of the area has a NB, 8.9% has a negative NNB balance or a NR, principally along rivers,
360 and 42.7% has a positive NNB or a NP, mainly located in agricultural areas.

361 To summarize, hot spots and low spots of NP and NR potentials can be distinguished in the
362 Garonne watershed. Riparian (streamside) areas and alluvial plains are the main hot spots of NP and NR
363 potentials for both landscapes (respectively Figure 4-1a and 4-1b for *Actual landscape* and Figure 4-2a

364 and 4-2b for *Natural landscape*). Low spots are also identified but they are different according to the
 365 function and the landscape under consideration. NP potential in the *Actual landscape* is low in Cambisols
 366 and areas covered by lawns. NP potential in the *Natural landscape* is low in areas with low soil organic
 367 matter content (lower than 2%). NR potential in the *Actual* and *Natural landscapes* is low in steep areas.
 368 The NNB maps give an idea of the net balance between NP and NR potentials. For the *Actual landscape*,
 369 the main net balance for NNB is production: hot spots of NP are located in agricultural areas whereas hot
 370 spots of NR are located along the rivers and near the cities (Figure 4-1c). For the *Natural landscape*, the
 371 main net balance for NNB is NR: hot spots of NP do not exist whereas hot spots of NR are located along
 372 the rivers and in the piedmonts of the Pyrenees or Central Basin (Figure 4-2c). For both scenarios, the
 373 most common potential is equal to -1 for NR, to +1 for NP and, to 0 for NB.



374
 375 *Figure 4: Disagreement maps (3x) in the Garonne basin between Actual (1x) and Natural landscapes (2x) for (a) Nitrate potential*
 376 *removal (NR); (b) Nitrate potential production (NP); (c) Nitrate Net Balance potential (NNB) (color should be used).*

3.2. Nitrate Net Balance (NNB) disagreement

Figure 4-3abc depicts the disagreement maps of NP, NR and NNB potentials. In Figure 4-3a, 56.7% of the watershed shows a disagreement for NP potential between *Actual* and *Natural landscapes*, including 42.5% of the area that is submitted to an increase of NP going from *Natural* to *Actual landscapes*. In the same way, Figure 4-3b shows that 57.6% of the watershed is in disagreement for NR and 46.3% of the area has potentially a loss of NR going from *Natural* to *Actual landscapes*. The NNB potential disagreement is the difference between NNB in *Natural* and *Actual landscapes* (Eq. 4). Finally, 66.8% of the basin area is submitted to a disagreement concerning NNB (Figure 4-3c). The disagreement varies from -9 to +9. A NNB potential disagreement above 0 indicates that there is a reduction of the nitrate-related ecological functions (NEF), leading to an increase of nitrate concentration for the *Actual landscape*. In other words, there is a “loss” of NEF from *Natural* to *Actual landscapes*. Three types of explanations can be listed: (1) an increase of NP, (2) a decrease of NR or (3) a loss of function (NR area became NP area). Some losses of NEF can be found near river networks, in agricultural areas and in lands having lost their natural characteristics, especially wetlands and forests (such as in the Massif Central). Conversely, a disagreement below 0 shows a gain of NEF potential from *Natural* to *Actual landscapes*. The area characterized by a gain of NEF is limited (6.4% of the basin area) and is located mostly in the Pyrenean mountain and urban areas. Areas without any change in NNB potential, representing 33.2 % of the basin area, are mostly located upland of the basin in the mountainous area where urban land uses are limited.

3.3. Validation of disagreement maps

In the agricultural site, the annual average denitrification rate was equal to $0.17 \mu\text{gN-N}_2\text{O}\cdot\text{h}^{-1}\cdot\text{gOM}^{-1}$ whereas, in the riparian forest area, the average rate was equal to $0.75 \mu\text{gN-N}_2\text{O}\cdot\text{h}^{-1}\cdot\text{gOM}^{-1}$ (Yao et al. 2017). In the agricultural area, our study simulated a NR potential of +3, +5 for NP and -2 for NNB whereas in the riparian forest the outputs were equal to +4 for NR, +3 for NP and -1 for NNB. Our model indicates that, in the riparian forest area, there is potentially a higher nitrate removal, confirmed by Yao et al. (2017) results.

The study of Pinay et al. (1995) showed the nitrate-related processes of two sites related to the Garonne riparian forest. The Verdun-sur-Garonne site has a higher rate for all processes: denitrification, mineralization and plant uptake, than in the Grenade site. The Verdun-sur-Garonne site retains more than 50% of the area N input whereas the Grenade site retains only 20% (Pinay et al. 1995), self-purification potential is higher in Verdun-sur-Garonne site. The NR potential of Verdun-sur-Garonne (=+5) is higher than the Grenade's one (=+4), while the NP potential is higher in the Grenade area (=+4) than in Verdun-sur-Garonne (=+3). The Verdun-sur-Garonne site has a higher potential of removing nitrate than the Grenade area. To conclude, the NNB potential of the Verdun-sur-Garonne riparian zone is equal to -2 whereas the Grenade area has a neutral balance (NB=0) of NNB potential. The study of Pinay et al. (1995) corroborates these findings. The comparison of our results with the study of Pinay et al. (1995) showed

413 that our model can properly assess the NR, NP and NNB potential, even if very simple environmental
414 factors were taken into accounts, such as land cover (riparian forest), distance to the river (near the river)
415 and slope (under 0.1%).

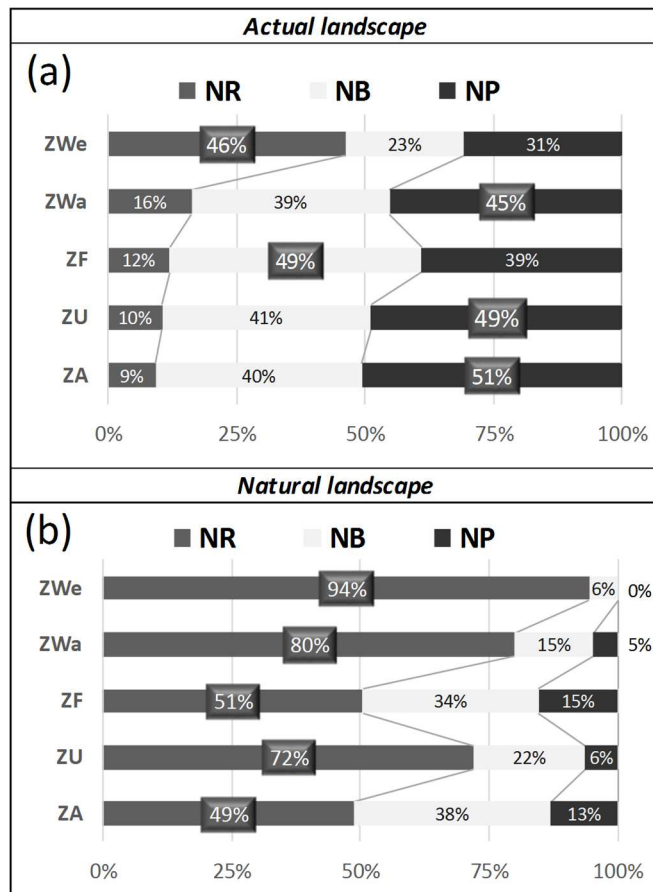
416 3.4. Factors affecting NNB potential and NNB disagreement

417 We compared the NNB potentials of both landscapes with the five input data used to build these
418 maps (Figure 4). For *Actual landscape*, Wetlands (ZWe, Table 3) are the most prevalent zone of NR
419 (37%), NB occurred mostly in Forests and semi-natural areas (ZF, 24%) and NP are appearing mostly in
420 Agricultural areas (22%). In *Actual landscape*, NR is disparate, mostly dominated by wetland presence,
421 which is in agreement with previous studies showing the benefits of wetland areas in NR functions
422 (Hansen et al., 2018; Montreuil et al., 2010; Sánchez-Pérez et al., 1991). For *Natural landscape*, the NR
423 function is homogeneous over zones with percentage varying between 14% (ZA and ZF) and 29% (ZWe).

424 Figure 5 summarizes, for both landscapes, the percentages of NR, NP and NB in each zone (see
425 zone definition in Table 3). For *Actual* NNB potentials, NR is stronger in wetlands (ZWe, 46%) compared
426 to the other land uses. NP occurs mainly in agricultural areas (ZA, 51%), artificial areas (ZU, 49%) and
427 water bodies (ZWa, 45%) (Figure 5-a). NP is also dominant in areas with low slopes (59.8% for slopes
428 under 9.6%) and low soil organic matter content (75.4% for soil with an organic matter content lower than
429 2%). However, NP has a stronger potential for land with an organic matter content higher than 5% (39%
430 of the total have a potential superior to +2), whereas NP areas with low organic matter content (lower than
431 2%) have 98% of this surface with a potential equal to +1 or +2.

432 For the *Natural* NNB potentials, 94% of the wetlands (ZWe) lead to NR, 6% to NB and 0% to NP
433 (Figure 5-b). Figure 5 shows a loss of NR function in the urban and agricultural areas (ZU and ZA) from
434 *Natural* to *Actual landscapes*. NR potential in ZU zone is 72% in *Natural landscape* whereas is reducing
435 to 10% in *Actual landscape* (Figure 5-b). In the same way, NR potential in ZA zone dropped from 49% to
436 9% from *Natural* to *Actual landscapes* (Figure 5-b). Simultaneously, NP potential drastically increases in
437 ZU and ZA areas. The human controlling to limit NP and remove nitrate through treatment plant
438 considered by the scientific expertise is not sufficient to overcome the loss of NEF.

439 The slopes do not appear to affect NNB. NR increases with distance from the river: 13% of the
440 area closest to the river show NR potential whereas 7% of the farthest class from the river show NR. In the
441 *Natural landscape*, 58% of the area with high soil organic matter content is characterized by NR potential.
442 Most of the potential vegetation classes are dominated by a NB function higher than 60%, excepted the
443 beech forest and the calcicolous mixed forest which are characterized mainly by NR for 62.4% and 47.4%,
444 respectively.



445
 446 *Figure 5: Percentage of the area occupied by each zones (Table 3) for Actual (a) and Natural landscapes, (c) and percentage by*
 447 *ecological function (NR, NB and NP) for Actual (b) and Natural landscapes (d) with NR: Nitrate Removal, NB: Neutral Balance*
 448 *and NP: Nitrate Production. The zonal repartition was determined by an overlay of the Actual and Natural land covers (Table 3).*
 449 *The dominant land covers of Actual landscape for each zone are Agricultural areas for ZA, Artificial areas for ZU, Forests and*
 450 *semi-natural areas for ZF, Water Bodies for ZWa and Wetlands for ZWe.*

451 Some relations between *Actual* input data and NNB disagreement can also be analyzed. The loss
 452 of NEF increases when the slope lowers, the distance to the river increases, or when the area is
 453 characterized by the development of agriculture. The first slope quartile has 68.6% of its surface
 454 characterized by NP and 57.8% for the fourth slope quartile. The loss of NEF is important in agricultural
 455 and artificial surfaces with respectively 67% and 60% of the total surface of the respective land use. The
 456 intensity of NEF loss is the lowest in wetland areas (27%). The gain of NEF is the highest in water bodies
 457 and wetlands with 30% and 27% of the surface, respectively. However, 51% of the wetland areas are
 458 characterized by a loss of NEF between *Natural* and *Actual landscapes*. 42.9% of the forest surface area
 459 does not present any change in NNB, 49.4% of the forest has a loss of NEF and only 7.9% has a gain of
 460 NEF.

3.5. Management application of potential disagreement maps

461 To understand potential management applications, we analyzed the NNB potential disagreement at
 462 the subbasin scale which is the scale used by water agencies in France and the other European countries
 463 applying the European Water Framework Directive (WFD). Figure 6 shows the majority class by
 464 subbasins for NNB potentials maps of both landscapes (Figures 6-1a and 6-2a) and the NNB disagreement
 465

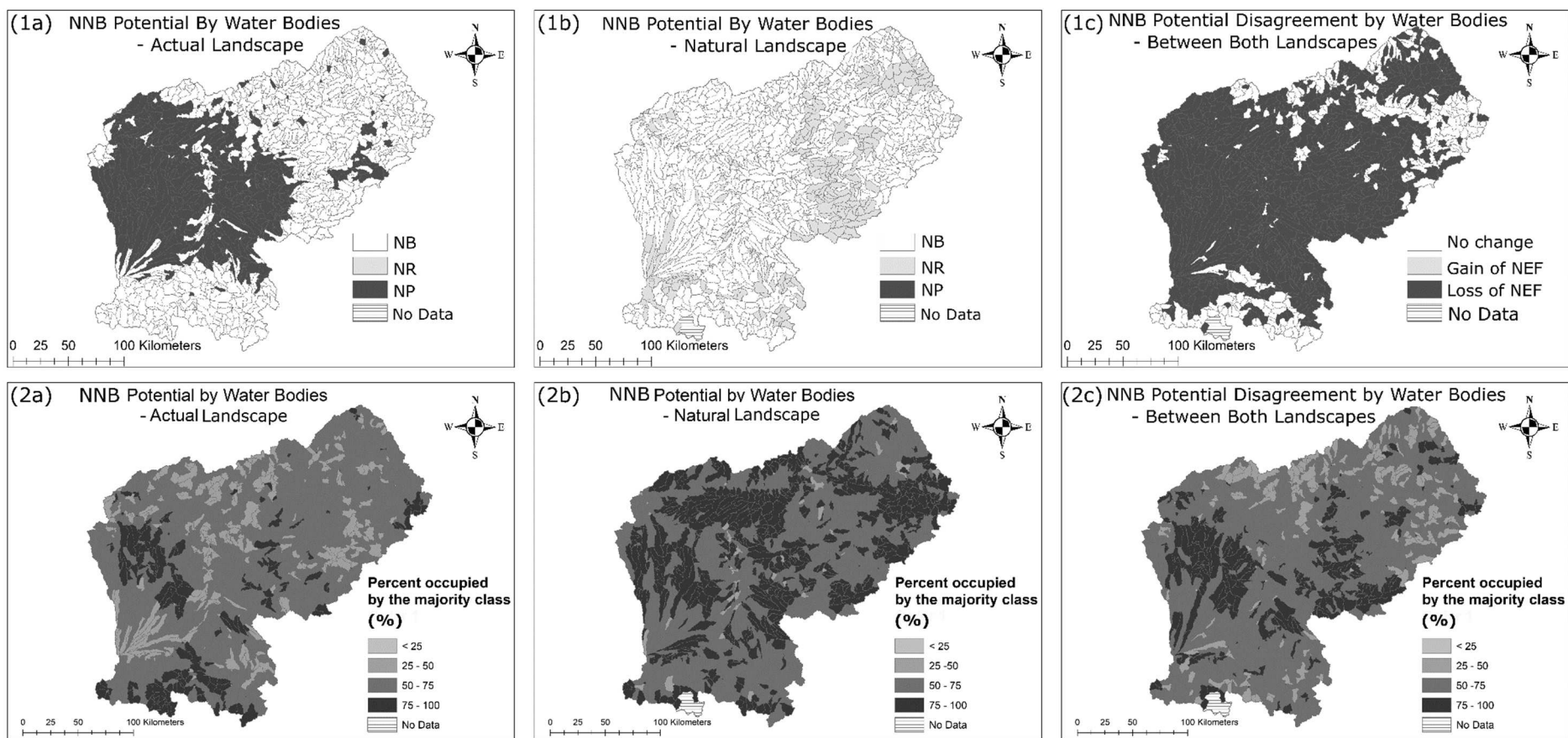
466 map (Figure 6-3a). The percentage occupied by the majority class in a given subbasin is generated from
467 the number of pixels in the majority class of the subbasin divided by the total number of pixels of the
468 subbasin. The percentage of area occupied by the majority class is given as an indication of the certitude
469 of the information provided (Figures 6-1b, 6-2b and 6-3b).

470 For the *Actual landscape*, 60% of NB occurring in the Garonne watershed is the majority class of
471 the subbasins whereas 38% of the NP corresponds to the majority class of the subbasins (Figure 6-1a). NR
472 is rarely the majority class and mostly the less frequent class (88% of the time). The average surface
473 occupied by the majority class is 62.4% of the subbasin areas with a standard deviation of 13.4% (Figure
474 6-2a). The second majority class occupies on average 29.3% of the subbasin areas with a standard
475 deviation of 11.1%. Finally, the less frequent class covers 8.3 % of the subbasin surface on average with a
476 standard deviation of 6.5%.

477 For the *Natural landscape*, there is no subbasin with a majority of NP (Figure 6-1b). The NP is the
478 less frequent class for 90% of the subbasins. In *Natural landscape*, 77% of the subbasin are occupied in
479 majority by NB functions and 23% by NR functions over the Garonne watershed (Figure 6-1b). The
480 subbasins with a majority of NR surface are located upland and in the Massif Central and Montagne Noire
481 areas. The majority class of the *Natural landscape* occupies on average 75% of the subbasins' surface
482 (Figure 6-2b) with a standard deviation of 15%.

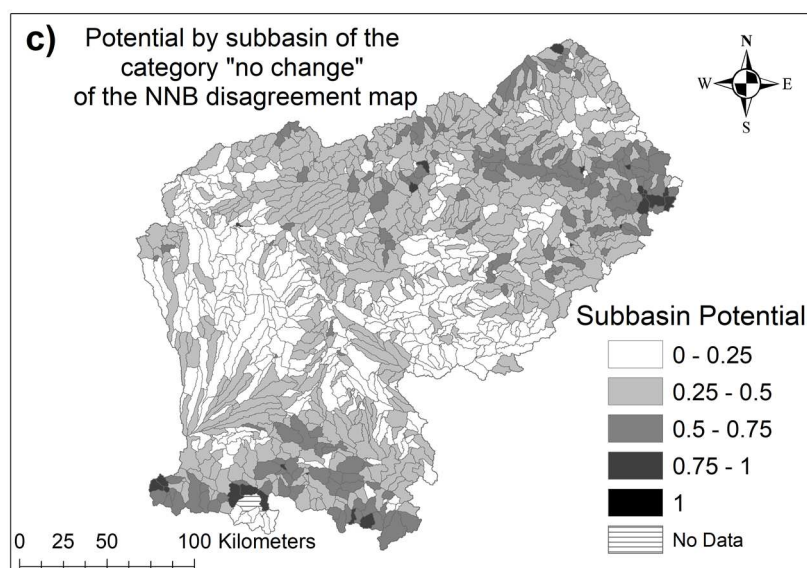
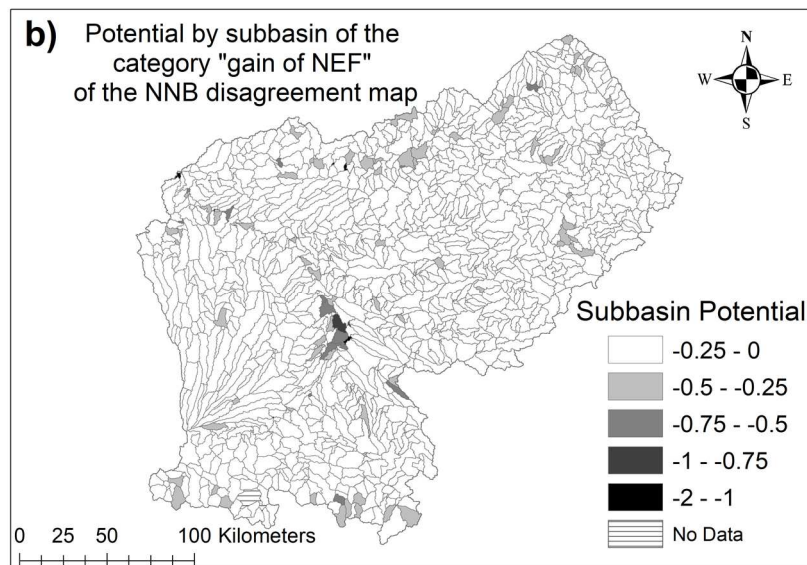
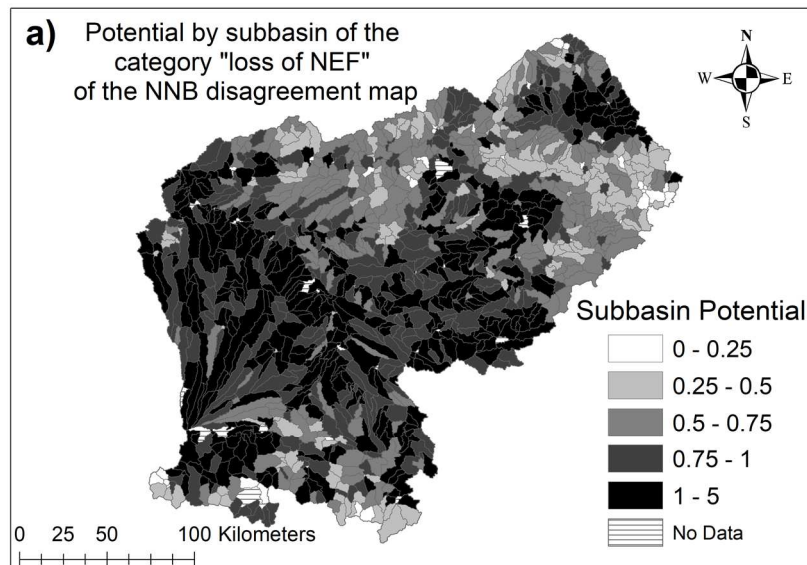
483 For the NNB disagreement map (Figure 6-1c), the most frequent disagreement occurring in
484 subbasins is the loss of NEF (76%). Only 6 subbasins out of 1309 have a gain of NEF in the majority. The
485 less frequent class is dominated by the gain of NEF (97 %). The average subbasin surface occupied by the
486 majority class is 65.7% ($\pm 14.5\%$) of the subbasin surface (Figure 6-2c) whereas the average surface
487 occupied by the less frequent class is 6.2% ($\pm 5.8\%$) of the subbasin surface. The impact of humans is
488 shown in Figure 6-1c where a majority of the watershed is characterized by a loss of NEF.

489 The subbasin potential (Eq. 4) of the loss is high in most of the subbasins (Figure 7-a) with an
490 average loss equal to 0.94 whereas the gain of NEF is a minority with an average potential of -0.1. The
491 subbasin potential of the loss is higher especially in the agricultural areas whereas the subbasin potential
492 of the gain is higher around the city of Toulouse and in the mountainous areas (Figure 7-b). The subbasin
493 potential of no-change category (average potential equal to 0.33) is strong in steep areas near the
494 mountains and the lowest in the plain areas.



495

496 Figure 6: Nitrate Net Balance (NNB) potential and disagreement class calculated as the majority class in each subbasin (1x) and the percentage of the area occupied by this majority class (2x).
 497 (1a) The majority class of NNB potential of Actual landscape with three types of functions: Neutral Balance (NB, white), Nitrate Removal (NR, grey) and Nitrate Production (NP, black). (1b)
 498 The majority class of NNB potential of Natural landscape with the same three types of functions. (1c) The majority class of NNB disagreement with three types of functions: No change (white),
 499 gain of NEF (Nitrate-related Ecological Function, grey) and loss of NEF (black). The percentage of the area occupied by the majority NNB class for Actual landscape (2a), for Natural
 500 landscape (2b) and for the NNB potential disagreement map (2c). The percentage occupied by the majority class in a given subbasin is generated from the number of pixels in the majority class
 501 of the subbasin divided by the total number of pixels of the subbasin.



502

503

504

Figure 7: Subbasin potential of (a) loss, (b) gain and (c) no change classes of Nitrate-related ecological function (NEF) deduced from the Nitrate Net Balance (NNB) disagreement maps between Actual landscape in each subbasin.

505 4. Discussion

506 4.1. Uncertainties of the multi-criteria decision analysis

507 The choice of the input data and the attribution of scores provided in Table 2 are based on
508 expert knowledge and published literature (Bernard-Jannin et al., 2015; Pinay et al., 2007; Sánchez-
509 Pérez et al., 1991; Yao et al., 2017). As an example, Hansen et al. (2018) and Sánchez-Pérez et al.
510 (2003) focused on wetlands' importance in the nitrogen cycle. In the same way, the role of organic
511 matter into the nitrate-related ecological function was emphasized in Hill et al. (2000) and Sánchez-
512 Pérez et al.(2003). The importance of land slope and distance to the river were also highlighted in
513 Macary et al. (2013). The experts involved in the study assumed that all chosen input datasets were
514 crucial in the estimation of NNB potential. The NR, NP and NNB potential classes have a normal
515 distribution for each scenario which confirmed the choice of equal weight. In the end, the output maps
516 may or may not follow the specialists' expectations. These maps are made to initiate discussion and
517 debate about the ecosystem functioning, the ecology, the impact of humans on nature and especially
518 on NNB potential. The methodology is easily repeatable and could be helpful to set up a rational
519 debate on an environmental issue where different experts participate (stakeholders, politicians,
520 environmental associations...). This type of methodology can be needed to rethink the interaction
521 dynamics between people and their environment.

522 Nitrogen cycle processes are still difficult to model at the watershed scale. For example, Valé
523 (2006) showed that mineralization processes vary greatly between soils, depending on irrigation
524 management and rainfall. The qualitative approach of the multi-criteria decision analysis shows some
525 advantages. This technique integrates some descriptive variables such as land use but also other
526 variables known to be important for nitrate dynamics. It is commonly used as a decision support tool
527 and the methodology and final diagnostic are easily understandable. This methodology is based on
528 "decision weights". This raises the question of how people transform probabilities into decision
529 weights. Research on this question is ongoing (Durbach and Stewart, 2012) and the comparison of
530 studies with different decision weights is very limited (Mavsar et al., 2014). Guitouni and Martel
531 (1998) provided guidelines to help in the choice of the analytic method. The methodology in this study
532 used only one weight and criteria combination allowing us to compare the different resulting maps
533 without any parameter maximization. The input data weighting had been decided by the analytic
534 hierarchy process (Saaty, 1990). The maps are potential maps based on scientific expertise and give an
535 idea of the possible tools that can be used by decision-makers. This study is in its early stage and the
536 results still need to be validated in further research with more data. The results of this study encourage
537 scientists to validate these results with in-situ measurements of NP, NR and NNB.

538 This work is built on the assumption that N loss and N gain can appear in the same area.
539 Therefore, this study is static explaining the duality between N loss and N gain. In a microcosm in
540 anaerobic conditions, both nitrification and denitrification processes can happen (Pihlatie et al., 2004;

541 Yang et al., 2019). However, when the N balance is considered in the system, the processes are not
542 simultaneous but appear one after another. The present study used simple variables to qualify N
543 processes. However, these processes are complex (Ajami and Gu, 2010; Martínez-Espinosa et al.,
544 2021) and a lot of variables affect NR and NP as microbial communities, soil texture, COD/N ratio,
545 *etc.* This simplification makes the model easily accessible and replicable but ignores the complexity of
546 some processes. The readers must be alarmed on that point. For future applications, this methodology
547 allows the user to adapt input variables to improve process quantification. Further studies will also
548 consider this model in dynamic using SWAT model and satellite image by running the model each
549 month or each year.

550 4.2. Spatial influence on the NNB potentials and disagreement

551 The NP and NR potential scores attributed to each input data are critical for the output maps of
552 Figure 4. However, NP and NR potential maps seem to give results consistent with the actual scientific
553 knowledge. Alluvial floodplains, in particular wetlands, are the most active places where a lot of
554 processes happen, including NR (Bernard-Jannin et al., 2015; Burt et al., 1999; Vidon et al., 2010).
555 Agricultural areas in the *Actual landscape* produce more nitrate and have a low NR potential (Billen et
556 al., 2013; Schnitzler-Lenoble and Carbiener, 2007). Mountainous areas do not appear to have the
557 highest NP and NR potentials (Figure 4-1c). However, when we focus on the NNB potential in the
558 *Natural landscape*, mountainous areas show the most important NR (Figure 4-2c).

559 The *Natural landscape* maps give an overview of the NNB “true” potential without any
560 human impacts. As expected, the *Natural* NNB potential map is covered mostly by NR and NB areas.
561 NR in the *Natural landscape* occurred mostly near the river networks and in wetland areas (Figure 4-
562 2c).

563 When we look at the *Actual* NNB map (Figure 4-1c), NP is the most represented function in
564 the basin and mostly in agricultural and urban areas (Groffman et al., 2004; McDonnell et al., 2008;
565 Powlson, 1993). In the mountainous area, the proportion of NB and NR is higher because these areas
566 have few human stressors (Figure 4-1c). This study shows that human stressors can be easily shown to
567 decision-makers through easily accessible data. By assessing the potential of self-regulating ecosystem
568 functions in *Natural landscape*, it is possible to determine the maximal Subbasin potential of these
569 NNB services in a non-anthropized watershed. When comparing these ecological function potentials
570 estimated in the *Natural landscape* with the *Actual landscape*, it makes it possible to quantify the
571 potential changes. These changes generated by the difference between these two landscapes (with and
572 without human stressors) referred as “NNB potential disagreement” in this study.

573 The mapping of the NNB disagreement should highlight the areas where human activities have
574 an impact on nitrate-related ecological functions and help evaluating the degree of human impact on
575 the landscape. Figure 4-3a shows the difference between *Actual* and *Natural* NP potentials. Upland

576 areas near the Pyrenees and Massif Central mountains are characterized by a majority of no change in
577 NP potential (Figure 4-3a), explained by low anthropogenic stressors. Upland areas of *Actual*
578 *landscape* are close to the upland areas of *Natural landscape*. For the NP potential (Figure 4-3a),
579 14.2% of the basin area has a higher NP potential in *Natural landscape* than in *Actual landscape*. This
580 loss of NP potential can be explained by high NP potentials in *Natural landscapes* allowed by the
581 presence of “active” wetlands, activating NP, whereas these places are occupied by deciduous forest
582 which activates NR in the *Actual landscape*. Moreover, urban areas in the *Actual landscape* are
583 considered as restricted areas, i.e. places without any processes. In the restricted areas, the difference
584 between *Actual* (restricted – no process) and *Natural landscapes* (existing potential production) results
585 in a loss of NP. Agricultural areas are characterized by a gain of NP caused by fertilizer inputs (Ratel
586 and Debrieur, 1992), whereas urban areas show a NP potential because of the city’s effluents but also
587 a NR potential thanks to wastewater treatment plants. Finally, urban areas also show a loss of NP
588 potential due to man-made purification systems used to remove pollutants from the system (Ratel and
589 Debrieur, 1992).

590 NNB potentials estimate in mountains are different according to the type and age of
591 mountains. The Pyrenees are a young mountainous massif with alpine and snowy stages (> 2,200 m
592 altitude), characterized by no vegetation and no processes due to high altitude whereas Massif Central
593 and Montagne Noire are the oldest massifs with only subalpine stage (<2,200m). The impact of
594 anthropization will be significant in the oldest massifs with changes in land cover and land use
595 practices, which shows Figure 6-1c with the highest disagreement in these oldest massifs. The North-
596 East and the East part of the watershed in the Massif Central and Montagne Noire are characterized by
597 a high loss of NR in areas where wetlands and forests disappeared between *Actual* and *Natural*
598 *landscapes* (Figure 4-2b). According to Figure 4-3b, the gain of NR happens mostly in the alluvial part
599 of the basin. One hypothesis is that the urban areas in this study are considered as areas with NR
600 potential thanks to the wastewater treatment plants, which have a water cleaning power, especially via
601 the denitrification process.

602 A positive NNB balance represents a NP of the system, which can lead to nitrate runoff and
603 diffuse pollution (Figure 4-3c). Figure 4-3c gives the NNB potential disagreement map showing that
604 human stressors have an impact on these functions in the entire basin. Human stressors not only affect
605 the land use but also the soil organic matter content (Campbell et al., 1991) and the presence of
606 wetland areas (Lomnický et al., 2019). The impact of human stressors on the different landscapes
607 along the watershed, in particular on the alluvial floodplain, is known since Antiquity (Lévêque,
608 2019). These human stressors increased in the XIX^e century with the setup of major hydraulic works
609 (Schnitzler-Lenoble and Carbiener, 2007). The Garonne basin and its river network are now highly
610 altered (Dynesius and Nilsson, 1994) as shown in Figure 4-3c. There is a high loss of NEF - meaning
611 an increase of NP or a diminishing of NR potential from *Natural* to *Actual landscapes* - in the riparian

612 forest (Figure 4-3c) where the forest management leads to massive forest clearing and to the
613 aggravation of alluvial loads. Scientific knowledge and literature results substantiate the results of this
614 study.

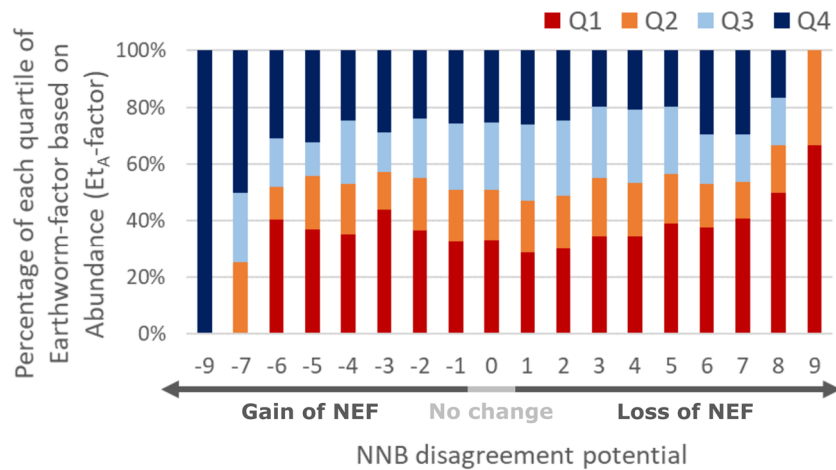
615 4.3. The uses of the factors affecting the NNB in environmental management application

616 As described previously in section 3.4, factors – slope, wetland, soil organic matter content,
617 land use and distance to the river - affect NNB. Land use choices impacts NNB potential. Agricultural
618 practices will bring N to the land increasing NP potential and will degrade soil biodiversity. Forests
619 and wetlands areas will increase NNB potential thanks to important soil biodiversity, presence of
620 organic matter with litter and the anaerobic conditions in soil which initiate denitrification processes.
621 Figure 5 shows that the land use changing have a direct impact on the NNB with a reduction of NR
622 potential and an increase of NP with the development of urban and agricultural area instead of forest
623 covers. For both landscapes, wetlands and riparian forests are hot spots of NR potential but the
624 disagreement on these areas indicates a high loss of NEF from *Natural* to *Actual landscape* correlated
625 with the loss of wetlands and riparian forests surfaces. In *Natural landscape*, 0.5% of the wetlands
626 zone (ZWe, Table 3) present NP potential whereas 31% of the wetlands (ZWe, Table 3) produce
627 nitrate in the *Actual landscape* (Figure 5). Human stressors have an impact on the NNB in wetlands,
628 areas which are supposed to be “natural”. These results look logical considering that reducing the
629 floodplain has increased human stressors on wetlands (Tockner and Stanford, 2002). Moreover, 79%
630 of the floodplains in Europe are intensively cultivated and the riparian forest lost 95% of its area and
631 around 40 to 70% of its ecosystem services (Schnitzler-Lenoble and Carbiener, 2007). The loss of
632 ecosystem services is induced by the reduction in water resource availability, the stoppage of the
633 natural nutritional inputs, the decline of native and sensitive species at the expense of the development
634 of aggressive non-indigenous species (Bravard et al., 1986). These losses of ecosystem services due to
635 human management endangers the population in particular with the increase of flood risks. Nowadays,
636 more and more initiatives are being taken to restore natural environments, particularly wetlands with a
637 flood protection role. These initiatives start with the need for nature of the local population which
638 implies the management of natural spaces.

639 Slopes and distance to rivers do not affect NNB but influence NR and NP potential. Therefore,
640 close to the river in alluvial plains, NP and NR potentials are higher than in steep areas. It is explained
641 by the highest probability to find anaerobic conditions in soil and longer residence time of N
642 compounds in soil (less run-off), leaving more time for N processes to occur. Slope and distance to the
643 river are not alterable on the short term at the basin scale, but stakeholder management can potentially
644 influence other inputs such as wetlands presence and land use. In the long term, the soil organic matter
645 content is also influenced by human practices through cultural practices (rotation, tillage, cover crops,
646 etc.) (Chenu et al., 2019). NP potential is stronger in areas with high organic matter content and areas
647 with low organic matter content are dominated by low NP potential (< +2). Agricultural areas are

648 characterized by low organic matter content and fertilizer application brings nitrates to soil
649 contributing to the NP potential. Understanding the impact of different management scenarios on the
650 NNB potential could allow providing a guideline for new studies in some areas. The organic matter
651 content is high near rivers in the riparian forest with the presence of litter and high velocity of litter
652 mineralization in soil. The surface of riparian forests is more important in *Natural landscape* than in
653 *Actual landscape*. The same methodology could be carried out again with other input datasets such as
654 the real vegetation type. Some studies showed the impact of management on the forest architecture
655 and the slowdown of the forest, liveliness inducing a loss of NEF (Bravard et al., 1986; Pautou et al.,
656 2003; Schnitzler-Lenoble and Carbiener, 2007). As an example, some deciduous trees such as alder,
657 beech or fruit trees, resist drought thanks to their deep root systems, contrary to the Salicaceae family
658 or white poplar which are more sensitive species (Pautou et al., 2003).

659 We compared the NNB disagreement potential with one indicator of soil erosion, the
660 earthworm factor (based on the earthworm abundance), evaluated at the European scale by internal
661 research activities in the European Soil Data Centre (JRC, Ispra). The earthworm factor is one
662 indicator of soil quality, a proxy of soil erosion, among others (Fu et al., 2019; Panagos et al., 2015;
663 Sharpley and Williams, 1990). The methodology used to determine the earthworm factor is explained
664 in Orgiazzi and Panagos (2018) and Panagos et al. (2014). The earthworm factor varies from 0 to 1, 1
665 indicating soils with a maximum abundance of earthworms. Figure 8 represents the average value of
666 the earthworm factor for each NNB disagreement class. In the Garonne basin area, the earthworm
667 factor varies from 0.737 to 0.999 and its variability is low. However, the earthworm factor is
668 increasing when the disagreement potential indicates a gain of NEF, whereas the earthworm factor is
669 the lowest when the loss of NEF is maximum (NNB disagreement = 9). NEF classes having a
670 significant impact on N production and removal are classes with high potential (below -5 or above
671 +4). These extreme classes gathered many environmental characteristics (OM, pH, land use, wetlands)
672 that make these areas with high potential for N production or N removal. These areas with high
673 potential are correlated with the presence of earthworm for a gain of NEF (higher N production or
674 removal) and the earthworm absence for loss of NEF (lower N production or removal). The
675 disagreement map seems to be correlated to this indicator of soil biodiversity. Indeed, a loss of NEF
676 will have a negative consequence on biodiversity abundance in terrestrial ecosystems. This effect has
677 been pointed out by Orgiazzi and Panagos (2018). A loss of biodiversity will induce a loss of NEF and
678 an increase of the disagreement potential. The comparison of our results with the earthworm factor
679 shows some correlations that supports our model results. However, further research on this topic is
680 encouraged.



681 Figure 8: Percentage of the soil biodiversity indicator, the earthworm factor (based on earthworm abundance), in each
 682 Nitrate Net Balance (NNB) disagreement class over the Garonne basin. The earthworm factors were divided in 4 groups
 683 according to the quartile repartition (Q1:[0.737 , 0.940]; Q2: [0.940 , 0.971]; Q3: [0.971 , 0.998]; Q4: [0.998 , 1]). NNB
 684 disagreement classes below 0 indicate a gain of Nitrate-related Ecological Functions (NEF), NNB disagreement above 0
 685 indicates a loss of NEF and a NNB equal to 0 indicates no change.
 686

687 4.4. Available options for the implementation of this model in water resource
 688 management decisions

689 This study aimed to evaluate the nitrate-related ecological functions potentials of the
 690 landscape based on datasets available in open-access. The results are convincing and we encourage
 691 further studies to test scenarios of landscape management to understand their effect on NNB potential
 692 and the evolution of the disagreement with the *Natural landscape*. The ideal management practices
 693 will bring Figure 6-1c closer to the natural state (Figure 6-1b). These studies might lead to provide
 694 management practices to preserve or improve the nitrate-related ecological functions of the watershed.
 695 Vrebos et al. (2015) used a similar spatial tool using scoring rules to assess ecosystem services and the
 696 effects of landscape on these services. These tools can be used to develop suitable management
 697 strategies in order to improve ecological functions evaluation and understanding behavior under
 698 human stressors and climate change.

699 The landscape NNB could also be compared to the assessment of NNB in the water system
 700 (Cakir et al., 2020) to understand if there is a direct or indirect relationship between land and river
 701 ecological functions. A lot of studies proved that there is a connectivity between land and in-stream
 702 processes (McMillan and Noe, 2017; Négrel et al., 2014), but the quantification of NNB potentials of
 703 each compartment (Sauvage et al., 2018) could provide some additional information on the spatial
 704 repartition of these functions and their potential relationships. Having a better understanding of the
 705 nitrogen cascade from land to the sea and especially on their nitrate removal / potential production
 706 could enable to set up some recommendations to supply food to the human population while
 707 preserving water resources (Billen et al., 2013).

708 5. Conclusion

709 The combination of a multi-criteria analysis of nitrate-related ecological function potential
710 with an evaluation of the nitrate-related ecological function disagreement confirms that the Garonne
711 basin is submitted to a high level of human stressors. 76% of the subbasins might lose nitrate-related
712 ecological function due to anthropogenic stressors, especially in agricultural and urban areas. Some
713 environmental factors influence the nitrate-related ecological function potential such as land use,
714 organic matter content and slope. Nitrate production is dominant in flat agricultural and artificial areas,
715 whereas wetlands and riparian forests boost the nitrate potential removal. Finding some management
716 practices to preserve or improve ecological functions in order to diminish the basin disagreement areas
717 could be a good way to help stakeholders in land management decisions and enhance awareness and
718 involvement of people for sustainable management.

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724 Authors contributions

725 R.C., S.S., R.W, M.Ge. and J.M.S.P. designed and developed the model. R.C., S.S., R.W,
726 M.Ge., E.R., M.Gu. and J.M.S.P. contributed to the paper analysis and discussions. R.C. wrote the
727 paper with considerable contributions from J.M.S.P, S.S., R.W, M.Ge., E.R., M.Gu. R.C reviewed the
728 paper with considerable contributions from S.S., E.R., and M.Gu

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