

SUPPLEMENTARY MATERIAL

Investigative monitoring of pesticide and nitrogen pollution sources in a complex multi-stressed catchment: the Lower Llobregat River basin case study (Barcelona, Spain)

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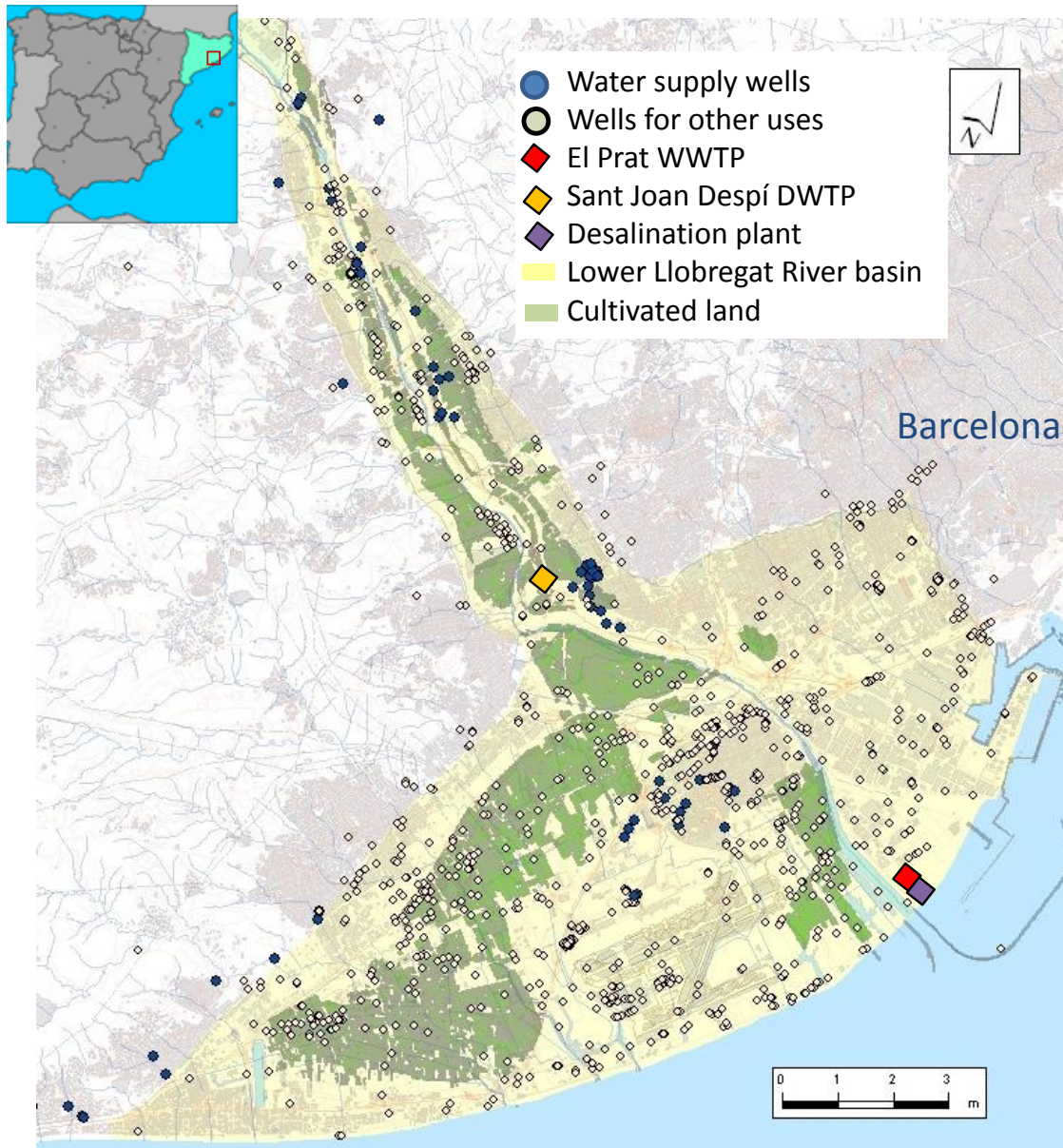


Figure S1. Map of the lower Llobregat River basin showing the location of water extraction wells, the main WWTP and DWTP, and the desalination plant.

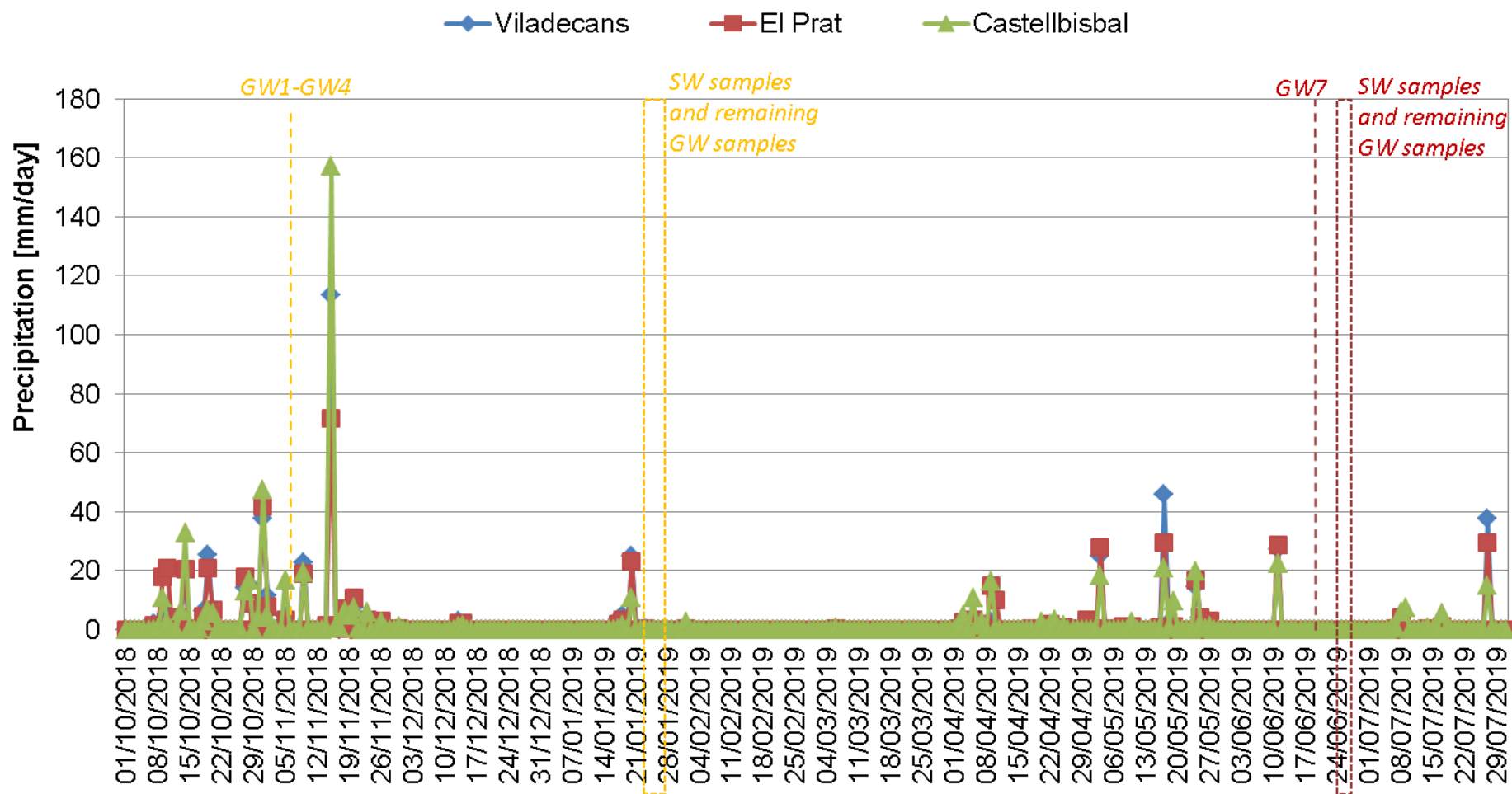


Figure S2. Precipitation (mm/day) registered in three stations located in the lower Llobregat River basin during the sampling periods (orange: winter campaign, red: summer campaign).

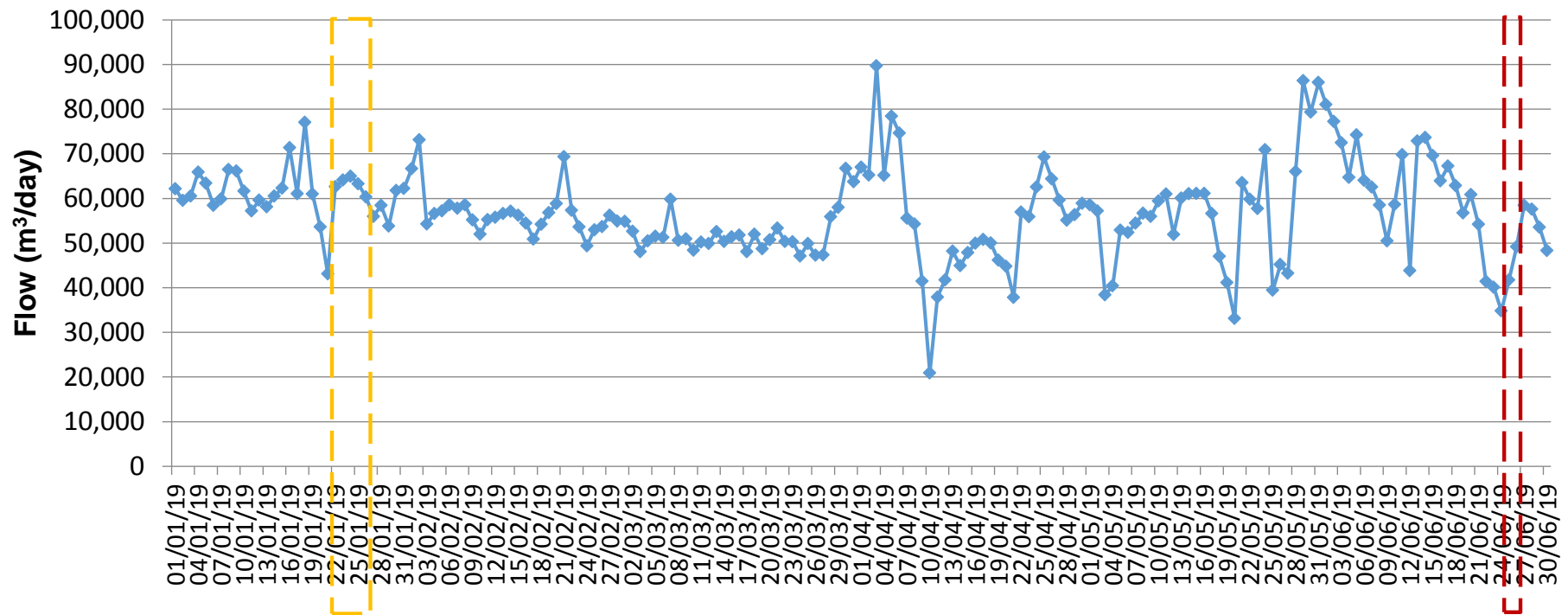


Figure S3. Daily average flow (m³/s) in the main Llobregat River (St Vicenç dels Horts gauging station) during the sampling periods (orange: winter campaign, red: summer campaign).

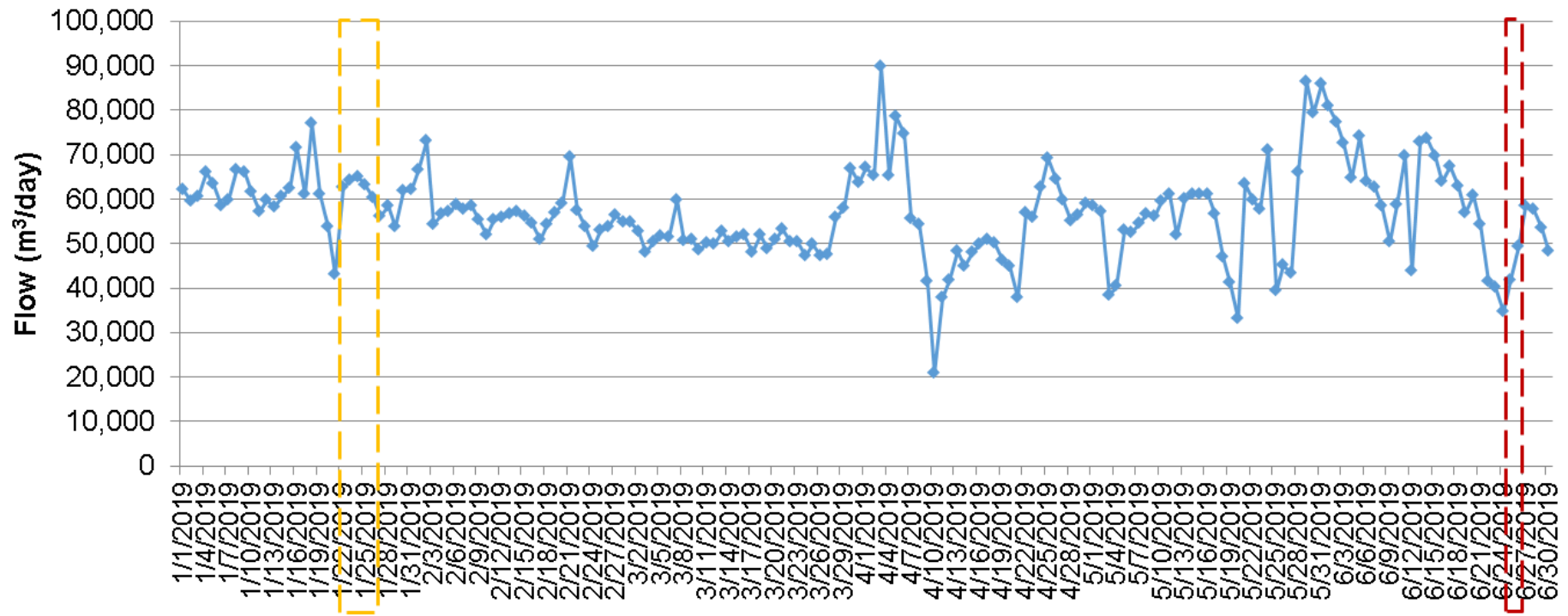


Figure S4. Daily average flow (m³/day) diverted from the Anoia tributary and the Riera Creek into the Infanta Channel irrigation network (SW3) (St Vicenç dels Horts gauging station) during the sampling periods (orange: winter, red: summer).

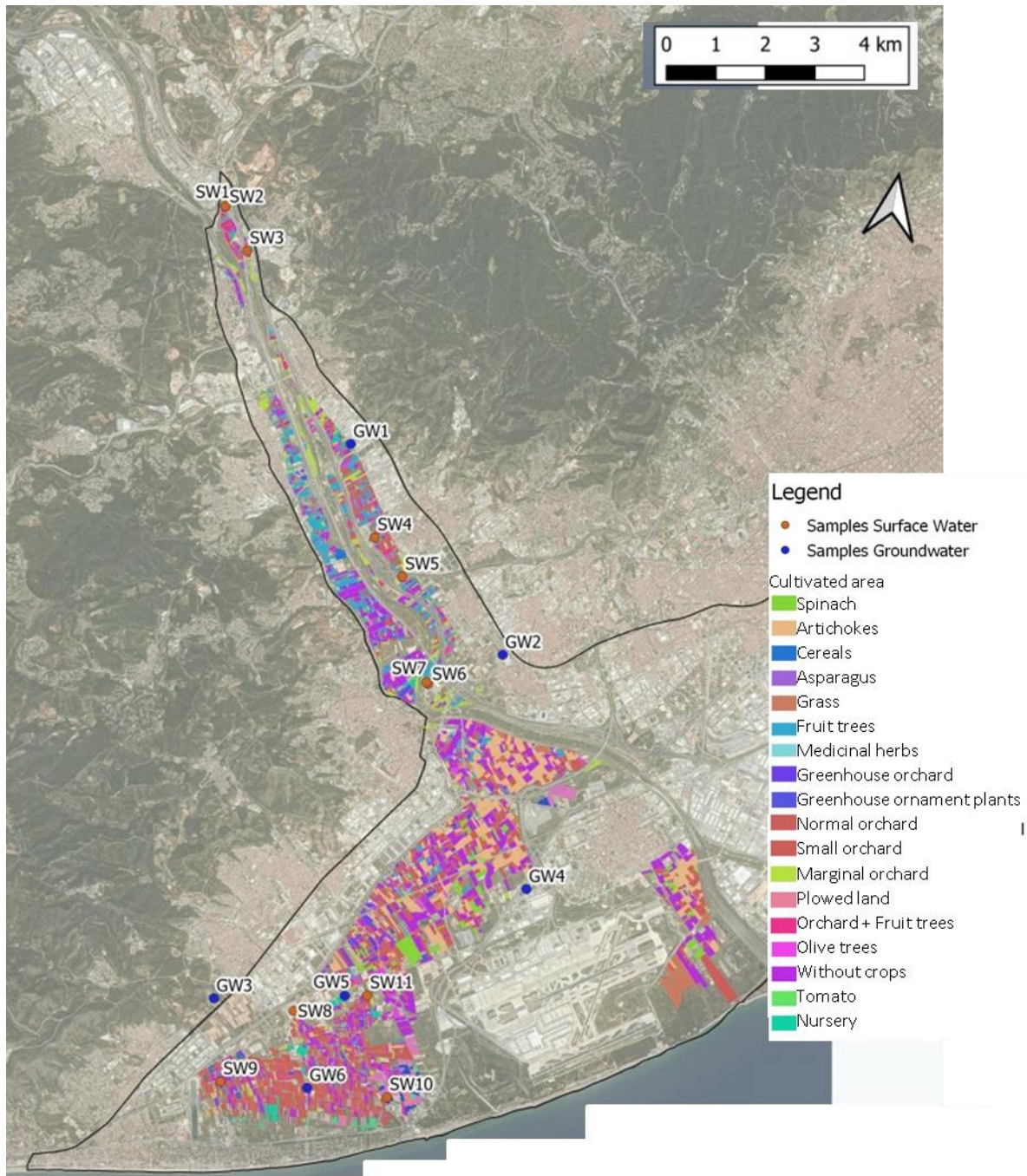


Figure S5. Map of the lower Llobregat River basin showing the high diversity of crops and fragmentation of cultivated land in the area.

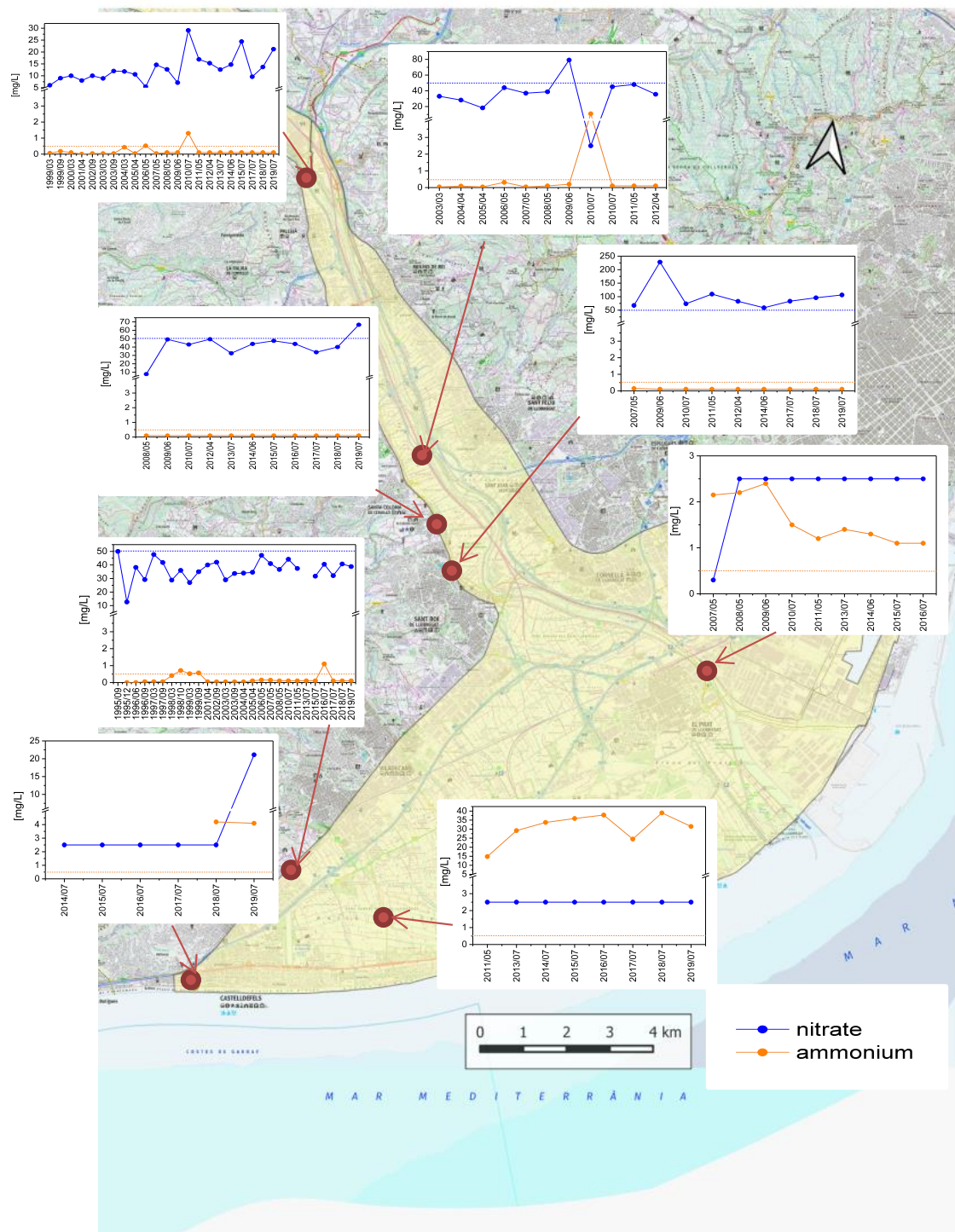


Figure S6. Historic pollution of nitrate and ammonium in the lower Llobregat River aquifer system. Water quality data obtained from the Catalan Water Agency (<http://aca-web.gencat.cat/sdim21/>).

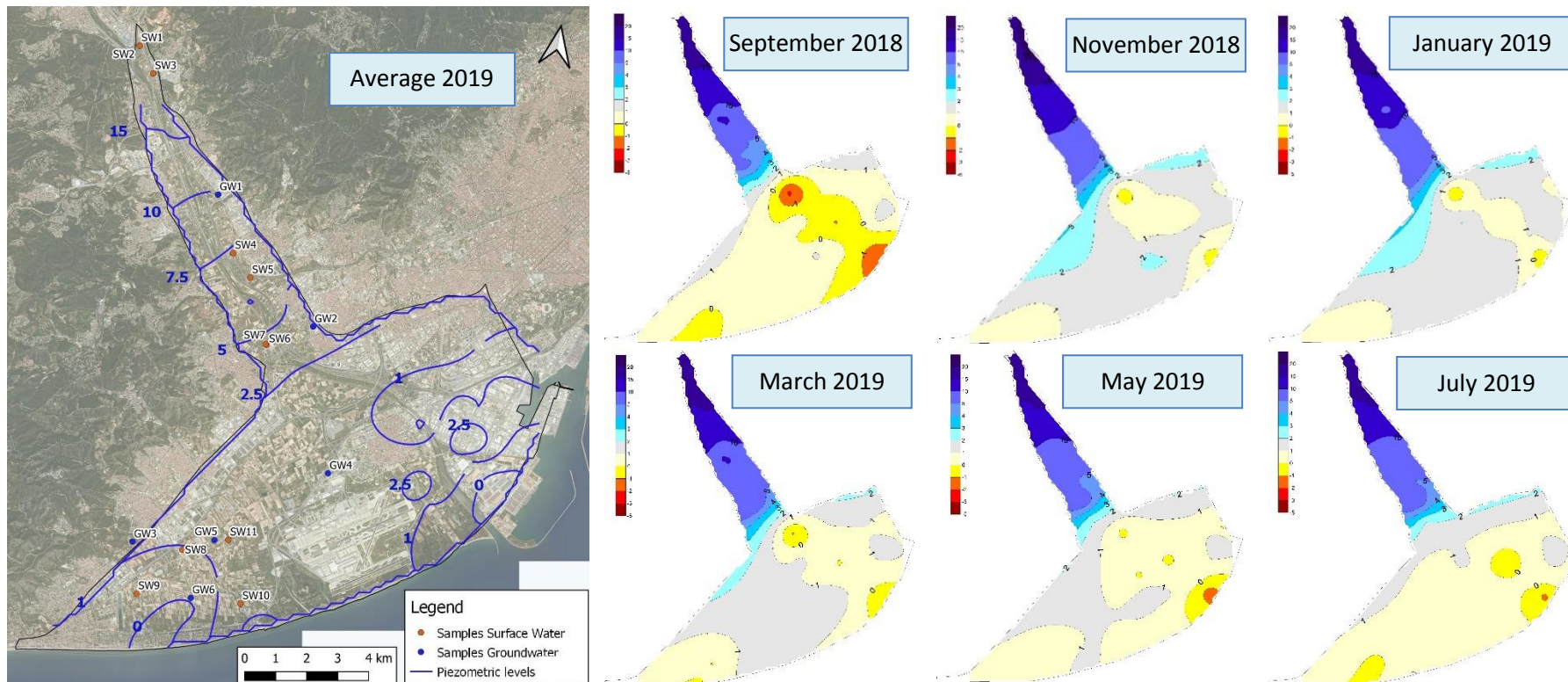
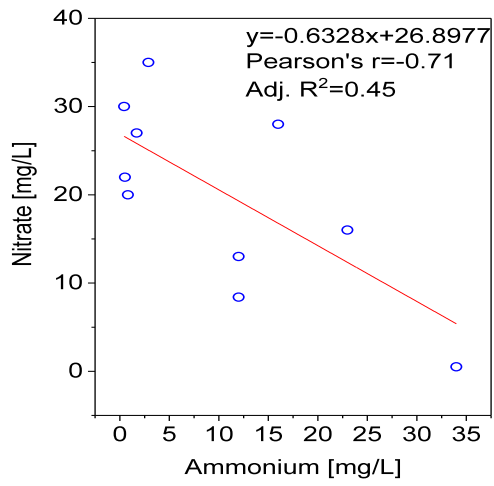


Figure S7. Piezometric levels (meters above sea level, masl) in the investigated area during the sampling period.

Winter



Summer

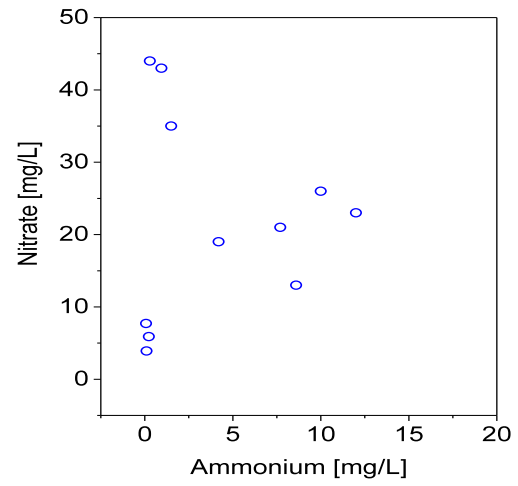


Figure S8. Correlation between ammonium and nitrate concentrations.

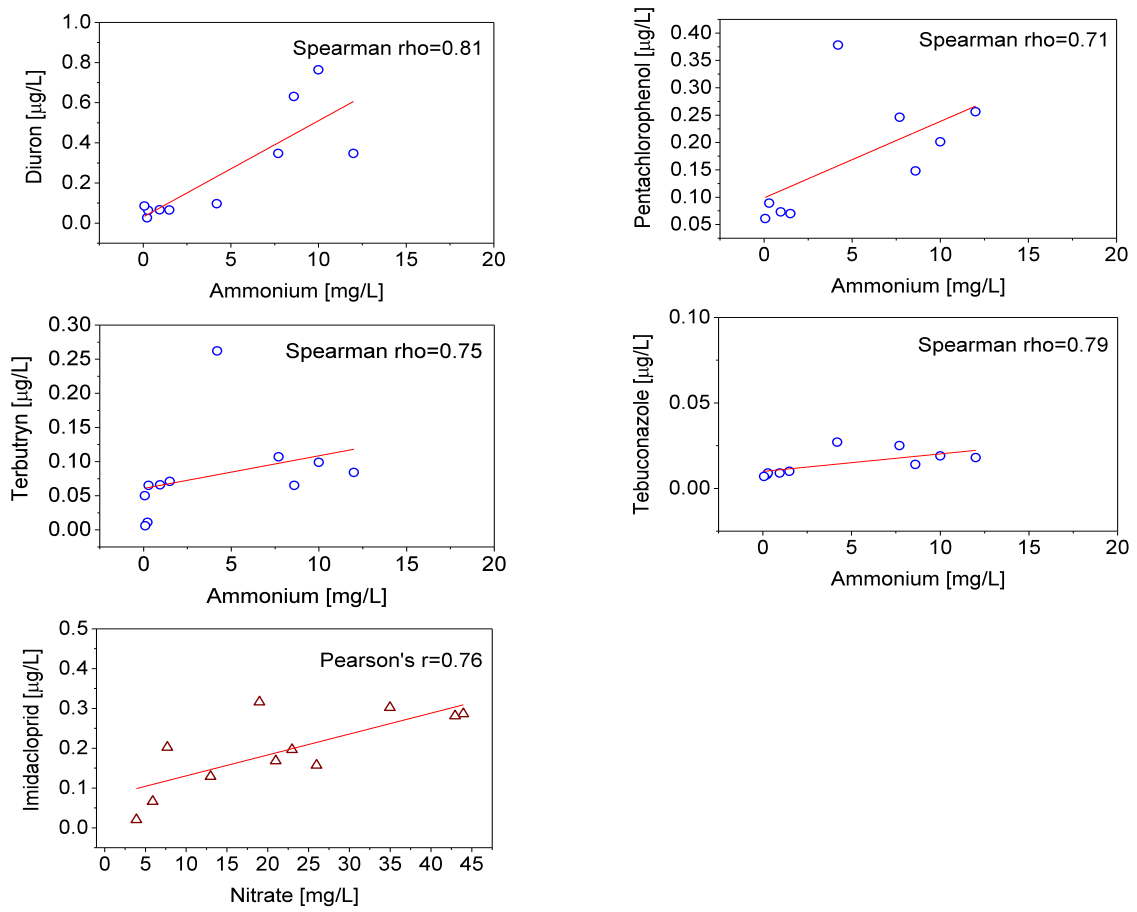


Figure S9. Significant correlations between ammonium or nitrate concentrations and individual pesticide concentrations. All corresponded to the summer period.

I. Interpretation of N, O, and B stable isotopic data: an overview

The isotopic composition of certain chemical compounds allows discriminating their origin (Aravena and Robertson, 1998; Clark, 1997). Moreover, depending on the processes undergone by the compound, the isotopic composition may vary and therefore also reveals the occurrence of processes, *e.g.*, the existence of natural attenuation (denitrification in the case of nitrate pollution).

¹⁵N and ¹⁸O in nitrogen-containing compounds

Nitrogen pollution can be related to sanitation network leakage, urban landfill leachate, and the use of synthetic fertilizers and/or manure. The analysis of the $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ of dissolved nitrate and $\delta^{15}\text{N-NH}_4^+$ of dissolved ammonium allows identifying to some extent the origin of these nitrogen compounds. Table S11 summarizes the expected δ values of the stable isotopes of N and O in nitrate and ammonium according to their origin.

Table S1. Stable isotope composition of N-compounds according to their origin

(Vitòria et al., 2004).

Origin	$\delta^{15}\text{N-NH}_4^+$	$\delta^{15}\text{N-NO}_3^-$	$\delta^{18}\text{O-NO}_3^-$
Fertilizers (inorganic)	- 5 ‰ - + 5 ‰	- 5 ‰ - + 5 ‰	+ 23 ‰.
Animal manure and urban wastewater (organic)	+ 8 ‰ - + 20 ‰	+ 8 ‰ - + 20 ‰	0 - + 7 ‰
Soil organic matter (organic)		+ 3 ‰ - + 9 ‰	
Nitrification			+1.7 ‰ - + 4.8 ‰

As shown in Table S11, nitrate from organic waste effluents present a $\delta^{18}\text{O-NO}_3^-$ lower than that of fertilizers, since it is generated by nitrification of ammonium or organic-bonded compounds. During this process, the oxygen incorporated in the nitrate molecule is derived from water oxygen and atmospheric oxygen (Mayer et al., 2001), and this allows discriminating between

fertilizers, and livestock/urban sanitation networks (see diagram $\delta^{15}\text{N-NO}_3^-$ vs. $\delta^{18}\text{O-NO}_3^-$ in Figure S8).

Nitrate derived from nitrification, either from inorganic ammonium fertilizers, from soil organic nitrogen, manure, or septic tanks and sewage networks, will have a characteristic $\delta^{18}\text{O-NO}_3^-$ that will be based on $\delta^{18}\text{O-H}_2\text{O}$ of local groundwater and $\delta^{18}\text{O-O}_2$ of oxygen dissolved in water (Mayer et al., 2001):

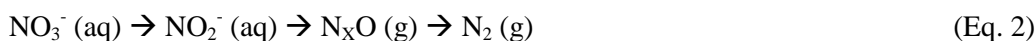
$$\delta^{18}\text{O}_{\text{NO}_3} = 1/3 \delta^{18}\text{O}_{\text{O}_2} + 2/3 \delta^{18}\text{O}_{\text{H}_2\text{O}} \quad (\text{Eq. 1})$$

where the value of $\delta^{18}\text{O-O}_2$ is + 23 ‰ and the value of $\delta^{18}\text{O-H}_2\text{O}$ is variable and depends on the study area. In Figure S8, the compositional boxes calculated with the minimum and maximum values of $\delta^{18}\text{O-H}_2\text{O}$ of groundwater of Mediterranean influence (Araguas-araguas, 2005) are presented.

Furthermore, when interpreting isotopic data of N compounds is also necessary to consider that the isotopic composition can be affected by various physical, chemical, and biological processes, such as volatilization, denitrification, mixing, etc.

Ammonium (NH_4^+), the main constituent of inorganic fertilizers, livestock manure, and wastewater, is affected by volatilization processes. Volatilization will produce an increase in $\delta^{15}\text{N-NH}_4^+$ (Mariotti et al., 1981). This process is represented in Figure S8 with a horizontal arrow.

One of the processes that can significantly modify the N and O isotopic composition of dissolved nitrate is denitrification (reduction of NO_3^- to N_2), natural attenuation of nitrate pollution. This reaction occurs through different intermediate stages.



This redox process acts under anaerobic conditions where bacteria obtain energy from the reduction of NO_3^- and the oxidation of organic matter or inorganic compounds such as sulfides (Rivett et al., 2008). During NO_3^- reduction, there is an isotopic fractionation that increases the

isotopic composition, both in $\delta^{15}\text{N-NO}_3^-$ and $\delta^{18}\text{O-NO}_3^-$ of the remaining nitrate with a characteristic slope (Böttcher et al., 1990; Fukada et al., 2003). Therefore, the use of stable isotopes is a very useful tool to determine the existence of denitrification processes since nitrate affected by denitrification will present an isotopic composition that will be projected on the theoretical field framed between these two bibliographic slopes. The variability of both slopes generates a zone of overlap or uncertainty where the origin of nitrate cannot be univocally assigned (Fig. S8).

Finally, in soils with high microbial activity, nitrate fertilizers can be recycled in the soil in a process abbreviated as MIT (Mineralization - Immobilization – Turnover), (Mengis et al., 2001). During this process, the isotopic composition of the N is approximately constant, but the $\delta^{18}\text{O}_{\text{NO}_3^-}$ loses its characteristic isotopic signal of + 23 ‰ and will have the same $\delta^{18}\text{O}_{\text{NO}_3^-}$ that nitrified ammonium-based fertilizers.

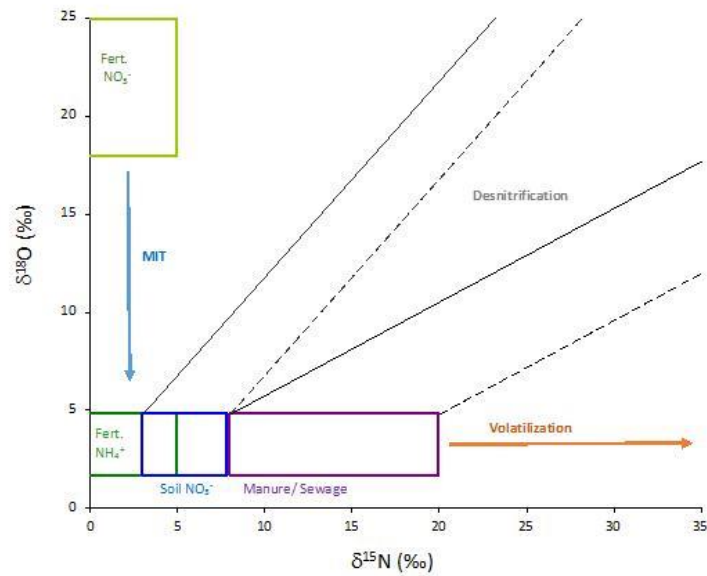


Figure S10. $\delta^{15}\text{N}\text{-NO}_3^-$ and $\delta^{18}\text{O}\text{-NO}_3^-$ showing the compositional boxes according to nitrate origin. Fertilizer data are from Vitòria et al. (2004), organic fertilizers and sewage data are from Widory et al. (2004), soil nitrate data are from Mengis et al. (2001). The $\delta^{18}\text{O}\text{-NO}_3^-$ of nitrification was calculated using the maximum and minimum $\delta^{18}\text{O}\text{-H}_2\text{O}$ values in the studied area (Eq. 1). Arrows indicate theoretical evolution during volatilization and MIT. The theoretical denitrification areas were calculated using published $\epsilon\text{N}:\epsilon\text{O}$ slopes of 2:1 (Böttcher et al., 1990) and 1.3:1 (Fukada et al., 2003). The range of $\delta^{18}\text{O}\text{-NO}_3^-$ theoretical values for nitrate from ammonium nitrification has been calculated using bibliographical values of groundwater of Mediterranean influence (Araguas-araguas, 2005), $\delta^{18}\text{O}\text{-H}_2\text{O}$ oscillates between -9.0 ‰ and -4.3 ‰, giving rise to values of $\delta^{18}\text{O}\text{-NO}_3^-$ between + 1.7 ‰ and + 4.8 ‰ (see Eq 1).

Boron

The values of $\delta^{11}\text{B}$ in natural waters (seawater, surface, groundwater, geothermal fluids, brines) have a range of values from -16 ‰ to + 60 ‰ (Tirez et al., 2010). This wide range allows the boron isotopic composition to be used as a tracer of pollutants in groundwater. The $\delta^{11}\text{B}$ of many contaminants such as wastewater, landfill leachate, and agricultural irrigation returns

show different isotopic compositions that can help to identify the source of contamination (Vengosh et al., 1994; Widory et al., 2005). Some studies have used boron isotopes as a tracer of landfill leachate in groundwater (Nigro et al., 2018). The reported values of urban waste landfill leachates range from -6.0 ‰ to + 25.1 ‰ (Barth, 2000; Hogan and Blum, 2003), different from the isotopic composition of the seawater boron (+ 39.5 ‰).

Water from wastewater treatment plants or industrial effluents greatly affects boron in groundwater and surface water (Chetelat and Gaillardet, 2005; Pennisi et al., 2006; Vengosh et al., 1994). However, Neal et al. (Neal et al., 2010) reported a decrease in boron content in rivers and wastewater treatment plant effluents. due to the substitution of percarbonates as a bleaching agent in soaps and detergents. At the same time, in some areas, boron derived from urban effluents has increased its $\delta^{11}\text{B}$ to values around +12 ‰ (Guinoiseau et al., 2018). That is why a good characterization of the boron derived from these effluents is necessary to correctly map out the contribution B of sources (Figure S9).

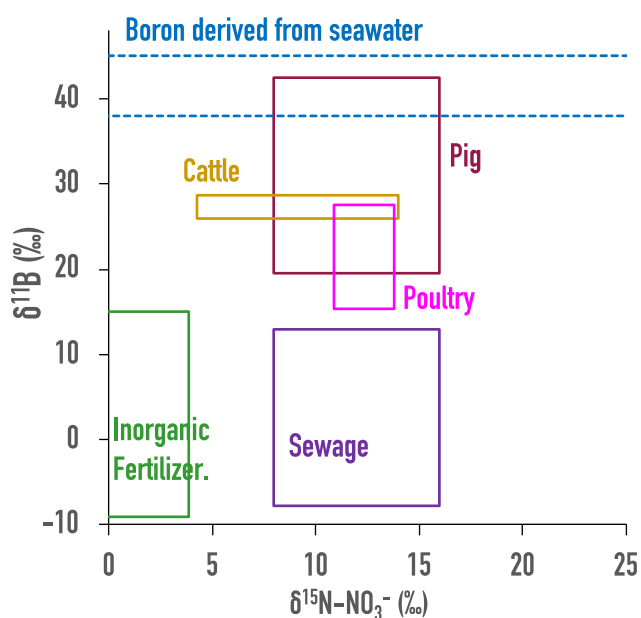


Figure S11. Graph of $\delta^{11}\text{B}$ vs. $\delta^{15}\text{N}$ with the various compositional boxes derived from bibliographic values (Seiler, 2005; Widory et al., 2004; Widory et al., 2005)

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