SUPPLEMENTARY MATERIAL

Environmental Risk Assessment of Neonicotinoids in surface water

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HPLC-MS-TQ method optimization and validation

Several mobile phases were tried (combining 2 mM ammonium acetate /0,1% formic acid with acetonitrile/methanol; Amulen et al., 2017; Elfikrie et al., 2020; Montiel-León et al., 2018, 2019; Sultana et al., 2018; Yi et al., 2019) being 2 mM ammonium acetate (A) and methanol (B) the solvents which gave the sharpest and more symmetrical shape of peaks. The optimized gradient program was: 5% of B for 10 s, after a linear gradient up to 98% of B in 15 min (kept 3 min) and finally came back to initial conditions (5% of B). Optimized MRM quantifier and qualifier transitions with their collision energies are listed in Table S2. NNIs identification and quantification were performed using isotopic dilution method, using acetamiprid-d3, clothianidin-d3, imidacloprid-d4 and thiamethoxam-d3 as extraction standards and thiacloprid-d4 as injection standard. Deuterated compounds were chosen because of their similar structure to the target analytes and they are not found in the environment. Calibration curves were generated using linear regression analysis over the established concentration range (1-1000 pg/µL). Intra-day precision (repeatability; CV<5%) essays were carried injecting nine times (n = 9) within the same day the same mix containing all the standards. Inter-day precision (reproducibility; CV<12%) was evaluated by injecting the solutions on nine non-consecutive days (n = 9). Field and laboratory blanks were extracted under the same conditions than the samples. Blank correction was applied where blank levels were above MDL. In addition, instrumental blanks consisting in acetonitrile were run before each sample injection to check cross-contamination possibility from HPLC-MS/MS system.

Table S1. CAS Nº, molecular weight, water solubility, lipophilicity (octanol-water coefficient, Kow), soil affinity (organic carbon-water partition coefficient, Koc), water hydrolysis, ecotoxicological data (EC50) and method detection limits (MDLs) for target NNIs.

	Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam
CAS number	135410-20-7	210880-92-5	138261-41-3	111988-49-9	153719-23-4
Molecular weight (g/mol)	227	250	256	270.74	293
Water solubility at 25ºC (mg/L) [1]	223	5997	7172	231.9	2862
Log Kow[2]	2.55	0.70	0.57	2.33	0.8
Log Koc[3]	2.84	1.43	1.53	1.05	0.85
BCFfish (L/kg w.w.) [4]	22.2	3.16	3.16	16.1	3.16
EC50 Algae (mg/L) [5] (Population abundance - 5 days)	1	55.0	10.0	60.6	81.0
EC50 Daphnia (mg/L) [6] (Intoxication and immobility - 48h)	49.8	119	85.0	22.5	100
EC50 Fish (mg/L) [7] (Multiple factors - NA)	100	100	211	25.2	100
LC50 *Americamysis bahía* (mg/L) [8] (Mortality 4 days)	6.60E-02	5.10E-02	3.80E-02	3.10E-02	6.90E+00
EC50 *Chironomus riparius* (mg/L)[9] (Acute test - 48h)	2.00E-02	2.20E-02	1.29E-02	1.80E-03	3.50E-02
EC50 *Chironomus dilutus* (mg/L)[10] (Emergence - NA)	7.00E-04	6.80E-04	2.40E-04	1.90E-04	1.30E-02
MDL (pg/L)	3.70	37.9	72.1	16.6	12.5

[1] Estimation programs Interface for Windows (EPI Suite; Experimental database match (EDM)); [2] Octanol-water coefficient (EPI Suite, KOWWIN-EDM); [3] Soil Adsorption Coefficient (EPI Suite, KOCWIN Kow Method); [4] Bioconcentration factor in fish (Log BCF Arnot-Gobas method (upper trophic)); [5,6,7,8] ECOTOX database; [9](Cavallaro et al., 2017)); [10](Raby et al., 2018).

Table S2. Instrumental parameters for target analytes by HPLC-MS/MS.

Compound	Precursor ion (m/z)	Quantitation ion (m/z)	Qualitative ion (m/z)	Quant. collision energy (V)	Qualit. collision energy (V)	Retention time (min)
Acetamiprid	223.1	126.1	56.1	15	10	7.05
Acetamiprid-d3	226.1	126.1	59.1	20	10	7.03
Clothianidin	250.0	250.0	169.0	10	20	6.91
Clothianidin-d3	253.0	172.0	209.1	10	10	6.90
Imidacloprid	256.0	209.0	175.0	10	20	6.76
Imidacloprid-d4	260.0	213.0	179.0	10	10	6.73
Thiacloprid	253.0	126.0	90.0	20	30	7.38
Thiacloprid-d4	257.0	190.0	215.0	10	10	7.35
Thiamethoxam	292.1	211.1	181.1	10	20	6.29
Thiamethoxam-d3	295.1	131.9	184.1	20	20	6.27

ESI (+) and capillary voltage 60 V.

Table S3. Neonicotinoid concentrations (ng/L) and statistical descriptives in surface waters.

	Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam	∑NNIs[1]
S1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
S2	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
S3	0.14	<MDL	5.06	<MDL	<MDL	5.06
S4	0.07	<MDL	<MDL	<MDL	<MDL	0.00
S5	<MDL	<MDL	0.87	<MDL	<MDL	0.87
S6	0.03	0.04	0.40	<MDL	0.06	0.46
S7	0.08	0.30	0.82	<MDL	<MDL	0.82
S8	0.12	<MDL	1.56	<MDL	<MDL	1.56
S9	0.05	0.05	0.28	<MDL	<MDL	0.28
S10	0.06	<MDL	1.15	0.04	0.04	1.22
S11	1.24	2.54	10.06	0.54	0.83	11.43
S12	2.82	<MDL	10.18	1.43	2.39	13.99
S13	<MDL	<MDL	6.27	<MDL	<MDL	6.27
S14	0.38	<MDL	2.35	<MDL	<MDL	2.35
S15	0.29	<MDL	3.09	0.42	<MDL	3.51
S16	<MDL	<MDL	3.34	<MDL	2.31	5.65
S17	<MDL	<MDL	1.24	<MDL	<MDL	1.24
S18	3.55	<MDL	5.44	2.56	<MDL	7.99
S19	0.13	<MDL	<MDL	<MDL	<MDL	0.00
Qf[2]	13/19	4/19	15/19	7/19	7/19	17/19
Median	0.07	0	1	0.0	0.01	1.3
Mean ± SD	0.47 ± 1	0.18 ± 0.58	2.75 ± 3.23	0.27 ± 0.65	0.3 ± 0.75	3.98 ± 5.17
Min-max	<MDL - 3.55	<MDL - 2.54	<MDL - 10.18	<MDL - 2.56	<MDL - 2.39	<MDL - 16.84

[1]Sum of acetamiprid, clothianidin, Imidacloprid, thiacloprid and thiamethoxam; [2] Quantification frequency (n>MDL/N).

Table S4. Comparison of imidacloprid concentrations (ng/L) in surface waters quantified in this study with previously published data.

Area	Country (water body)	Concentration (ng/L) Mean (median)	min-max	Qf[1]	Sampling year	Reference
Europe	Spain (Tagus river)	2.75 (1.24)	<MDL-10.2	15 (19)	2020	This study.
	Spain (Tagus river)	- (2.68)	<MDL-21.5	44 (48)	2018	Rico et al., 2018.
	Spain (Llobregat river basin)	-	2.5-134	11 (11)	Winter 2019	Postigo et al., 2021.
		-	20 - 218	11 (11)	Summer 2019	
	Spain (Ter river)	ND	ND	0 (11)	2018	Barbieri et al., 2020.
	Spain (Llobregat river)	19 (-)	<10-190	3 (11)	2017	
	Spain (Ebro River basin and Catalan River Basin)	32 (11)	164 (max)	58(89)	2020	Llorens et al., 2020.
	Spain (Llobregat river)	- (42.24)	<MDL-447	68 (97)	2016	Quintana et al., 2019.
		- (<LOQ)	<MDL-15	3 (62)		
	Spain (Jucar, Ebro, Llobregat and Guadalquivir rivers)	-	1.1-6.1	18 (35)	2013	Masiá et al., 2013.
	Spain (Ebro river)	1.8 (-)	<MDL-6.14	14 (24)	2010	Ccanccapa et al., 2016.
		2.2 (-)	<MDL-19.20	4 (24)	2011	
	Spain (Turia and Júcar rivers)	- (3.53)	<MDL-51.84	2 (15)	2010	Ccanccapa et al., 2016b.
		- (2.19)	<MDL-14.31	3 (15)	2011	
		- (23.12)	<MDL-206.96	18 (22)	2012	
		- (20.93)	<MDL-95	21 (29)	2013	
	Sweden (streams to the river Råån)	-	<MDL-15000	24 (68)	2008	Kreuger et al., 2010.
	Switzerland (Mentue, Limpach, Furtbach, Surb, Salmsacher Aach)	-	<MDL-9.2	14 (45)	2012	Moschet et al., 2014.
North America	USA (Aberjona river, Massachusetts)	-	<MDL-6.9	14 (118)	2009-2010	Wijnja, Doherty, & Safie, 2014.
	USA (seven stream basins within Iowa, Missouri, and Mississippi rivers)	- (<2)	<MDL-42.7	18 (79)	2014	Hladik et al., 2014.
	Canada (Saskatchewan wetlands)	-	<MDL-30.2	27 (136)	Spring 2012	Main et al., 2014.
		-	<MDL-256	11 (134)	Summer 2012	
		-	<MDL	0 (80)	Fall 2012	
		-	<MDL-4.8	2 (90)	Spring 2013	
	USA (Wood Creek, Salt Creek, Grayson Creek, Alamo	50 (-)	<MDL-160	26 (37)	2010	Ensminger et al., 2013.

	Creek, Pleasant Grove Creek and Alder Creek watersheds)	<MDL (-)	<MDL-670	19 (37)	2012	
	USA (Sope Creek) USA (Cattahoochee River)	- -	<MDL-35.5 <MDL-10.1	11 (13) 6 (10)	2011-2012	Hladik & Calhoun, 2012.
	USA (rivers from Salinas Valley, Santa Maria Valley and Imperial Valley)	- -	<MDL-3050 <MDL-3290	34 (39) 33 (36)	Fall 2010-Spring 2011 Summer 2010-Summer 2011	Starner & Goh, 2012.
	Canada (Gibeault-Delisle river)	1256 (150)	35-7770	7(7)	2005-2006	Gibeault-Delisle, 2010.
Oceania	Australia (river water from several creeks around Sydney)	200 (-)	<MDL-4560	14 (93)	2013	Sánchez-Bayo & Hyne, 2014.
Asia	Japan (Rivers and estuaries of Osaka city)	2.5 (-) 8.6 (-)	<MDL-7 <MDL-25	16 (24) 20 (21)	2009 2010	Yamamoto et al., 2012.
	Vietnam (Chieng Khoi watershed)	25 (-)	<MDL-1153	11 (24)	2008	Lamers et al., 2011.

Table S5 Comparison of acetamiprid concentrations (ng/L) in surface waters quantified in this study with previously published data.

Area	Country (water body)	Concentration (ng/L)		Qf[1]	Sampling year	Reference
		Mean (median)	min-max			
Europe	Spain (Tagus river)	0.47 (0.07)	<MDL-3.55	13 (19)	2020	This study.
	Spain (Llobregat river basin)	-	ND	0 (0)	Winter 2019	Postigo et al., 2021.
		-	<MDL - 28	3 (11)	Summer 2019	
	Spain (Ter river)	ND	ND	0 (11)	2018	Barbieri et al., 2020
	Spain (Llobregat river)	ND	ND	0 (11)	2017	
	Spain (Ebro River basin and Catalan River Basin)	2 (0)	110 (max)	3(89)	2020	Llorens et al., 2020
	Sweden (streams to the river Råån)	-	<MDL-410	13 (68)	2008	Kreuger et al., 2010.
North America	Canada (Saskatchewan wetlands)	- - - -	<MDL-5.2 <MDL-54.4 <MDL-12.0 <MDL	1 (136) 1 (134) 4 (80) 0 (90)	Spring 2012 Summer 2012 Fall 2012 Spring 2013	Main et al., 2014.
	USA (Iowa agricultural rivers)	-	<MDL -11.1	1 (79)	2014	Hladik et al., 2014.
Oceania	Australia (river creeks around Sydney)	80	<MDL-380	11 (73)	2013	Sánchez-Bayo & Hyne, 2014.
Asia	Japan (Rivers and estuaries of Osaka city)	<MDL 1.4	<MDL <MDL-1.4	0 (24) 2 (21)	2009 2010	Yamamoto et al., 2012.

Table S6. Spearman rank correlation matrix.

		Thiametoxam	Imidacloprid	Clothianidin	Acetamiprid	Thiacloprid	∑NNIs	SiD	H	pH	O_2 (mg/L)	O_2 (%sat)	Amonium	Fosfate	Nitrate
Thiametoxam		1													
	Sig. (2-tailed)	.													
	N	5													
Imidacloprid	Cor. Coef.	0.8	1												
	Sig. (2-tailed)	0.104	.												
	N	5	15												
Clothianidin	Cor. Coef.	1.000(**)	0.8	1											
	Sig. (2-tailed)	.	0.2	.											
	N	2	4	4											
Acetamiprid	Cor. Coef.	0.8	.918(**)	1.000(**)	1										
	Sig. (2-tailed)	0.2	0	.	.										
	N	4	11	4	13										
Thiacloprid	Cor. Coef.	1.000(**)	0.7	.	1.000(**)	1									
	Sig. (2-tailed)	.	0.188	.	.	.									
	N	3	5	1	5	5									
∑NNIs	Cor. Coef.	0.8	.986(**)	0.8	.813(**)	0.7	1								
	Sig. (2-tailed)	0.104	0	0.2	0.001	0.188	.								
	N	5	15	4	13	5	17								
SiD	Cor. Coef.	0.6	0.049	0.5	0.117	-0.4	-0.036	1							
	Sig. (2-tailed)	0.285	0.879	0.667	0.748	0.6	0.906	.							
	N	5	12	3	10	4	13	15							
H	Cor. Coef.t	0.6	-0.042	0.5	0.139	-0.4	-0.066	.970(**)	1						
	Sig. (2-tailed)	0.285	0.897	0.667	0.701	0.6	0.831	0	.						
	N	5	12	3	10	4	13	15	15						
pH	Cor. Coef.	0	-0.055	-0.5	-0.336	-0.1	-0.27	0.294	0.254	1					
	Sig. (2-tailed)	1	0.859	0.667	0.312	0.873	0.35	0.288	0.362	.					
	N	5	13	3	11	5	14	15	15	16					
O_2 (mg/L)	Cor. Coef.	0	0.077	0.5	0.205	0.205	-0.033	.684(**)	.693(**)	0.153	1				
	Sig. (2-tailed)	1	0.802	0.667	0.545	0.741	0.911	0.005	0.004	0.571	.				
	N	5	13	3	11	5	14	15	15	16	16				
O_2 (%sat)	Cor. Coef.	0.6	0.302	0.5	0.445	0.6	0.152	.689(**)	.704(**)	0.068	.925(**)	1			
	Sig. (2-tailed)	0.285	0.316	0.667	0.17	0.285	0.605	0.005	0.003	0.803	0	.			
	N	5	13	3	11	5	14	15	15	16	16	16			
Amonium	Cor. Coef.	0.4	-0.31	1.000(**)	-0.429	-0.5	-0.286	-0.228	-0.238	0.69	-.881(**)	-.810(*)	1		
	Sig. (2-tailed)	0.6	0.456	.	0.397	0.667	0.493	0.588	0.57	0.058	0.004	0.015	.		
	N	4	8	2	6	3	8	8	8	8	8	8	8		
Fosfate	Cor. Coef.	-0.3	-0.227	-0.5	-0.317	-0.2	-0.227	-.804(**)	-.745(*)	-0.145	-.647(*)	-0.573	0.524	1	
	Sig. (2-tailed)	0.624	0.502	0.667	0.406	0.747	0.502	0.005	0.013	0.67	0.031	0.066	0.183	.	
	N	5	11	3	9	5	11	10	10	11	11	11	8	11	
Nitrate	Cor. Coef.	0	0.25	1.000(**)	0.286	-0.5	0.25	0.393	0.393	-0.714	-0.25	0.071	-0.4	0.371	1
	Sig. (2-tailed)	1	0.589	.	0.535	0.667	0.589	0.383	0.383	0.071	0.589	0.879	0.6	0.468	.
	N	4	7	3	7	3	7	7	7	7	7	7	4	6	7

**Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

Table S7. Comparison of thiacloprid concentrations (ng/L) in surface waters quantified in this study with previously published data.

Area	Country (water body)	Concentration (ng/L)		Qf[1]	Sampling year	Reference
		Mean (median)	min-max			
Europe	Spain (Tagus river)	0.27 (0.01)	<MDL-2.56	7 (19)	2020	This study.
	Spain (Ter river)	ND	ND	0 (11)	2018	Barbieri et al., 2020.
	Spain (Llobregat river)	4.3 (-)	<0.79-31	3 (11)	2017	
	Spain (Ebro River basin and Catalan River Basin)	1 (0)	30 (max)	16(89)	2020	Llorens et al., 2020.
	Switzerland (Mentue, Limpach, Furtbach, Surb, Salmsacher Aach)	-	NA-65	16 (45)	2012	Moschet et al., 2014.
	Sweden (streams to the river Råån)	-	<MDL	0 (68)	2008	Kreuger et al., 2010.
Oceania	Australia (Sydney)	150	<MDL-1370	12 (80)	2013	Sánchez-Bayo & Hyne, 2014.

Table S8. Comparison of thiamethoxam concentrations (ng/L) in surface waters quantified in this study with previously published data.

Area	Country (water body)	Concentration (ng/L)		Qf[1]	Sampling year	Reference
		Mean (median)	min-max			
Europe	Spain (Tagus river).	0.30 (0.01)	<MDL-2.39	7 (19)	2020	This study.
	Spain (Ter river)	ND	ND	0 (11)	2018	Barbieri et al., 2020.
	Spain (Llobregat river)	ND	ND	0 (11)	2017	
	Switzerland. (Mentue, Limpach, Furtbach, Surb, Salmsacher Aach	-	NA-47	27 (45)	2012	Moschet et al., 2014.
North America	Iowa (USA); agricultural rivers	(<2)	<MDL -32.4	37 (79)	2014	Hladik et al., 2014.
	Saskatchewan. Canada; prairie wetlands	0.1 - - -	<MDL-144 <MDL-3110 <MDL-30.99 <MDL 173	14 (136) 25 (134) 5(80) 20 (90)	Spring 2012 Summer 2012 Fall 2012 Spring 2013	Main et al., 2014.
Australia	Sydney rivers (agricultural) (Australia)	100	<MDL-200	4 (27)	2013	Sánchez-Bayo & Hyne, 2014.
Asia	Rivers and estuaries of Osaka city; Japan	1.5 3.8	<MDL-25 1.5-11	18 (24) 21(21)	2009 2010	Yamamoto et al., 2012.
	Switzerland; Swiss plateau rivers	-	2.2-47	(27) 45	2012	Moschet et al., 2014.
	Sweden; horticultural crops river water	-	<MDL-15000	24 (68)	2008	Kreuger et al., 2010.

Table S9. Comparison of clothianidin concentrations (ng/L) in surface waters quantified in this study with previously published data.

Area	Country (water body)	Concentration (ng/L)		Qf[1]	Sampling year	Reference
		Mean (median)	min-max			
Europe	Spain (Tagus river)	0.73 (0.17)	<MDL-2.54	4 (19)	2020	This study.
	Spain (Ter river)	ND	ND	0 (11)	2018	Barbieri et al., 2020.
	Spain (Llobregat river)	ND	ND	0 (11)	2017	
	Spain (Ebro River basin and Catalan River Basin)	1 (0)	25 (max)	6(89)	2020	Llorens et al., 2020
	Switzerland; (Mentue, Limpach, Furtbach, Surb, Salmsacher Aach)	-	<MDL-4.4	1 (45)	2012	Moschet et al., 2014.
North America	Iowa (USA); agricultural rivers	(8.2)	<MDL -257	18 (79)	2014	Hladik et al., 2014.
	Saskatchewan. Canada; prairie wetlands	23.52 42.43 - -	<MDL-144 <MDL-3110 <MDL-30.99 <MDL 173	49 (136) 68.34 (134) 4 (80) 78 (90)	Spring 2012 Summer 2012 Fall 2012 Spring 2013	Main et al., 2014.
Australia	Sydney rivers (agricultural) (Australia)	60	<MDL-420	8 (53)	2013	Sánchez-Bayo & Hyne, 2014.
Asia	Japan	3.5 2.9	<MDL-1.4 <MDL-12	20 (24) 21 (21)	2009 2010	Yamamoto et al., 2012.

Table S10. Neonicotinoid mass flow rates (kg/y) in Tagus river watershed.

	Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiamethoxam	∑NNIs[1]
S1	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
S2	<MDL	<MDL	<MDL	<MDL	<MDL	<MDL
S3	0.01	<MDL	0.19	<MDL	<MDL	0.20
S4	0.05	<MDL	<MDL	<MDL	<MDL	0.05
S5	<MDL	<MDL	0.03	<MDL	<MDL	0.03
S6	0.01	0.01	0.07	<MDL	0.01	0.10
S7	0.04	0.14	0.39	<MDL	<MDL	0.58
S8	0.04	<MDL	0.56	<MDL	<MDL	0.60
S9	0.02	0.02	0.10	<MDL	<MDL	0.13
S10	0.00	<MDL	0.03	0.00	0.00	0.03
S11	0.83	1.71	6.75	0.36	0.56	10.20
S12	1.45	<MDL	5.22	0.73	1.23	8.63
S13	<MDL	<MDL	0.31	<MDL	<MDL	0.31
S14	0.03	<MDL	0.18	<MDL	<MDL	0.21
S15	0.00	<MDL	0.02	0.00	<MDL	0.02
S16	<MDL	<MDL	0.13	<MDL	0.09	0.22
S17	<MDL	<MDL	0.01	<MDL	<MDL	0.01
S18	1.13	<MDL	1.72	0.81	<MDL	3.66
S19	0.19	<MDL	<MDL	<MDL	<MDL	0.19
Median	0.01	0.00	0.10	0.00	0.003	0.20
Mean ± SD	0.20 ± 0.43	0.10 ± 0.39	0.83 ± 1.87	0.10 ± 0.25	0.10 ± 0.30	1.34 ± 2.97
Min-max	<MDL – 1.45	<MDL – 1.71	<MDL – 6.75	<MDL – 0.81	<MDL – 1.23	<MDL – 10.2

[1]Sum of acetamiprid, clothianidin, Imidacloprid, thiacloprid and thiamethoxam;

Table S11. Predicted environmental concentrations in the aquatic environmental ecosystem studied: predicted environmental concentration for fresh water (PECfw (mg/L)), predicted non effect concentrations for fresh water (PNECfw (mg/L)), risk characterization ratios for fresh water (RCRfw), risk characterization ratios for the mix based on PEC/PNEC relation (RCRmix(PEC/PNEC)), toxic units (TU), sum of toxic units (STU), and RCRmix (STU) on diferent trophic levels: algae-*Daphnia*-fish (a-d-f), algae- *Americamysis bahia*-fish (a-a-f), algae- *Chironomus riparius*- fish (a-*c.rip*-f), algae- *Chironomus dilutus*- fish (a-*c.dil*-f).

	Acetamiprid	Clothianidin	Imidacloprid	Thiacloprid	Thiametoxam	∑NNIs
PEC_{fw} (mg/L)[1]	3.55E-06	2.54E-06	1.02E-05	2.56E-06	2.39E-06	-
$PNEC_{fw}$ (mg/L)[2]	5.0E-04	1.3E-04	9.0E-06	5.0E-05	1.4E-04	-
RCR_{fw}	0.007	0.020	1.131	0.051	0.017	-
RCR_{mix}(PEC/PNEC)	-	-	-	-	-	1.23
TU (algae)	3.55E-06	4.62E-08	1.02E-06	4.22E-08	2.95E-08	-
STU (algae)	-	-	-	-	-	4.69E-06
TU (daphnia)	7.13E-08	2.14E-08	1.20E-07	1.14E-07	2.39E-08	-
STU (daphnia)	-	-	-	-	-	3.50E-07
TU (fish)	3.55E-08	2.54E-08	4.82E-08	1.02E-07	2.39E-08	-
STU (fish)	-	-	-	-	-	2.35E-07
$RCR_{mix(STUa-d-f)}$	-	-	-	-	-	0.005
TU (americanys)	5.38E-05	4.99E-05	2.68E-04	8.25E-05	3.46E-07	
STU (americanys)	-	-	-	-	-	4.54E-04
$RCR_{mix(STU\ a-a-f)}$	-	-	-	-	-	0.45
TU (c. riparius)	1.78E-04	1.16E-04	7.89E-04	1.42E-03	6.82E-05	-
STU (c. riparius)	-	-	-	-	-	2.57E-03
$RCR_{mix(STU\ a-c.rip-f)}$	-	-	-	-	-	2.57
TU (c. dilutus)	5.08E-03	3.74E-03	4.24E-02	1.35E-02	1.84E-04	
STU (c. dilutus)	-	-	-	-	-	6.49E-02
$RCR_{mix(STU\ a-c.dil-f)}$	-	-	-	-	-	64.9

[1]Maximun concentrations in surface water (Table S4); [2] (Carvalho et al., 2015).

Table S12. RCR_{mix} calculted as the ratio between PEC and PNEC and the sum of toxic units (STU) for the most sensitive trophic level: algae-*daphnia*-fish (a-d-f), algae-*Americamysis bahia*-fish (a-a-f), algae- *Chironomus riparius*- fish (a-*c.rip*-f), algae-*Chironomus dilutus*- fish (a-*c.dil*-f).

	RCRmix (PEC/PNEC)	RCRmix (STUa-*d*-f)	RCRmix (STUa-*a*-f)	RCRmix (STUa-*c.rip*-f)	RCRmix (STUa-*c.dil*-f)
S1	0.00	0.00	0.00	0.00	0.00
S2	0.00	0.00	0.00	0.00	0.00
S3	0.56	0.00	0.14	0.40	21.30
S4	0.00	0.00	0.00	0.00	0.10
S5	0.10	0.00	0.02	0.07	3.63
S6	0.05	0.00	0.01	0.04	1.80
S7	0.09	0.00	0.03	0.08	3.97
S8	0.17	0.00	0.04	0.13	6.68
S9	0.03	0.00	0.01	0.03	1.32
S10	0.13	0.00	0.03	0.11	5.07
S11	1.16	0.00	0.35	1.28	50.31
S12	1.18	0.00	0.36	1.79	54.14
S13	0.70	0.00	0.16	0.49	26.11
S14	0.26	0.00	0.07	0.20	10.33
S15	0.35	0.00	0.10	0.49	15.50
S16	0.39	0.00	0.09	0.32	14.09
S17	0.14	0.00	0.03	0.10	5.18
S18	0.66	0.00	0.28	2.02	41.19
S19	0.00	0.00	0.00	0.01	0.19

Table S13. Diversity indexes (SiD and H') and physical-chemical parameters from samples, when available

Sample	SiD	H'	pH	O2 (mg/L)	O2 (%sat)	Amonium	Fosfate	Nitrate
S1	0.89	3.66	8.15	8.97	90.44	<0,1	<0,1	<5
S2	0.73	2.37	8.12	8.70	89.84	<0,1	<0,1	<5
S3	0.78	2.73	8.30	8.92	86.60	<0,1	0.10	11.40
S4	0.78	2.67	8.10	8.70	87.40	<0,1	<0,1	<5
S5	0.79	3.03	7.58	9.90	90.40	<0,1	<0,1	<5
S6	0.73	2.07	7.97	8.03	78.40	0.71	0.59	15.00
S7	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
S8	0.63	1.72	7.98	8.04	82.42	<0,1	<0,1	6.24
S9	0.72	2.04	7.59	5.94	60.19	6.26	0.82	21.00
S10	0.69	1.96	7.75	7.39	78.86	0.32	1.83	37.40
S11	0.80	2.77	7.83	8.54	86.98	<0,1	0.12	43.40
S12	0.77	2.62	7.86	7.23	81.00	1.83	0.66	22.00
S13	0.40	1.42	7.44	7.56	80.00	0.13	1.31	<5
S14	0.78	2.99	7.13	8.58	88.00	0.12	0.14	<5
S15	N.A.	N.A.	7.69	7.23	74.66	<0,1	0.17	<5
S16	0.78	2.72	7.02	9.39	93.76	0.10	0.20	<5
S17	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
S18	0.45	1.25	6.94	8.22	83.14	0.17	0.39	<5
S19	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.

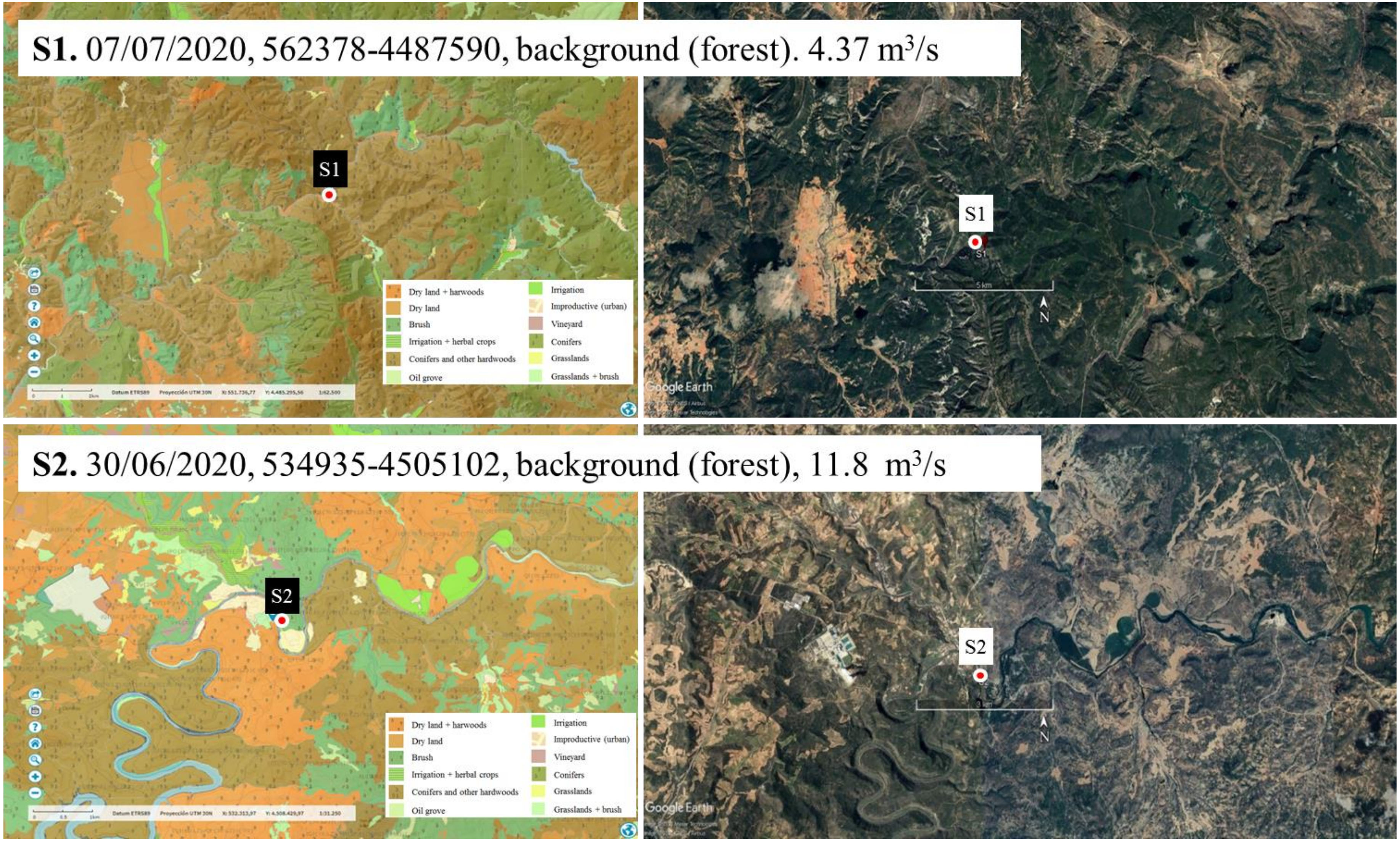


Figure S1 Continuation. Sample, date, location (UTMX-UTMY), soil characterization and water flow (m^3/s) at each sampling site.

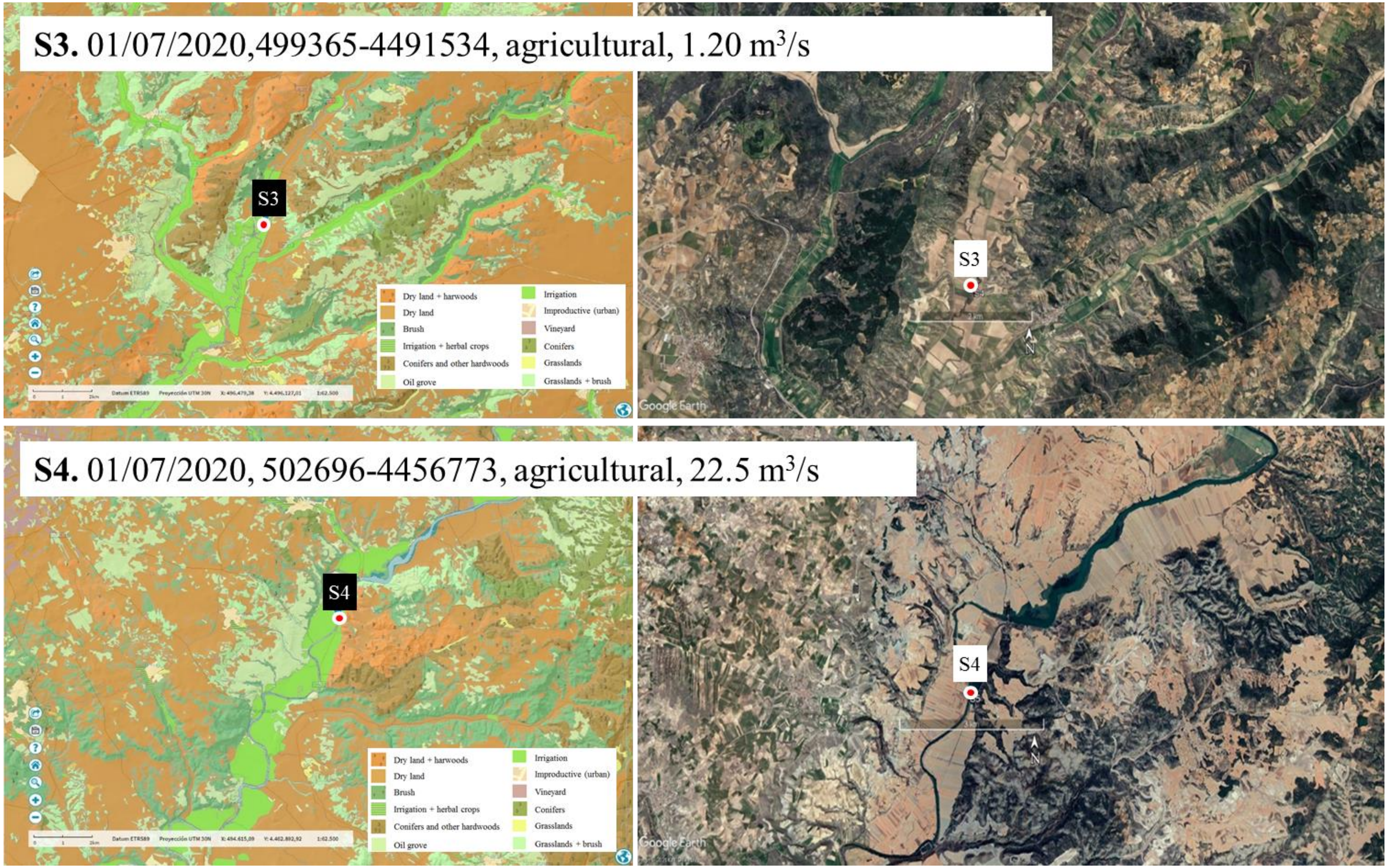


Figure S1 Continuation. Sample, date, location (UTMX-UTMY), soil characterization and water flow (m^3/s) at each sampling site.

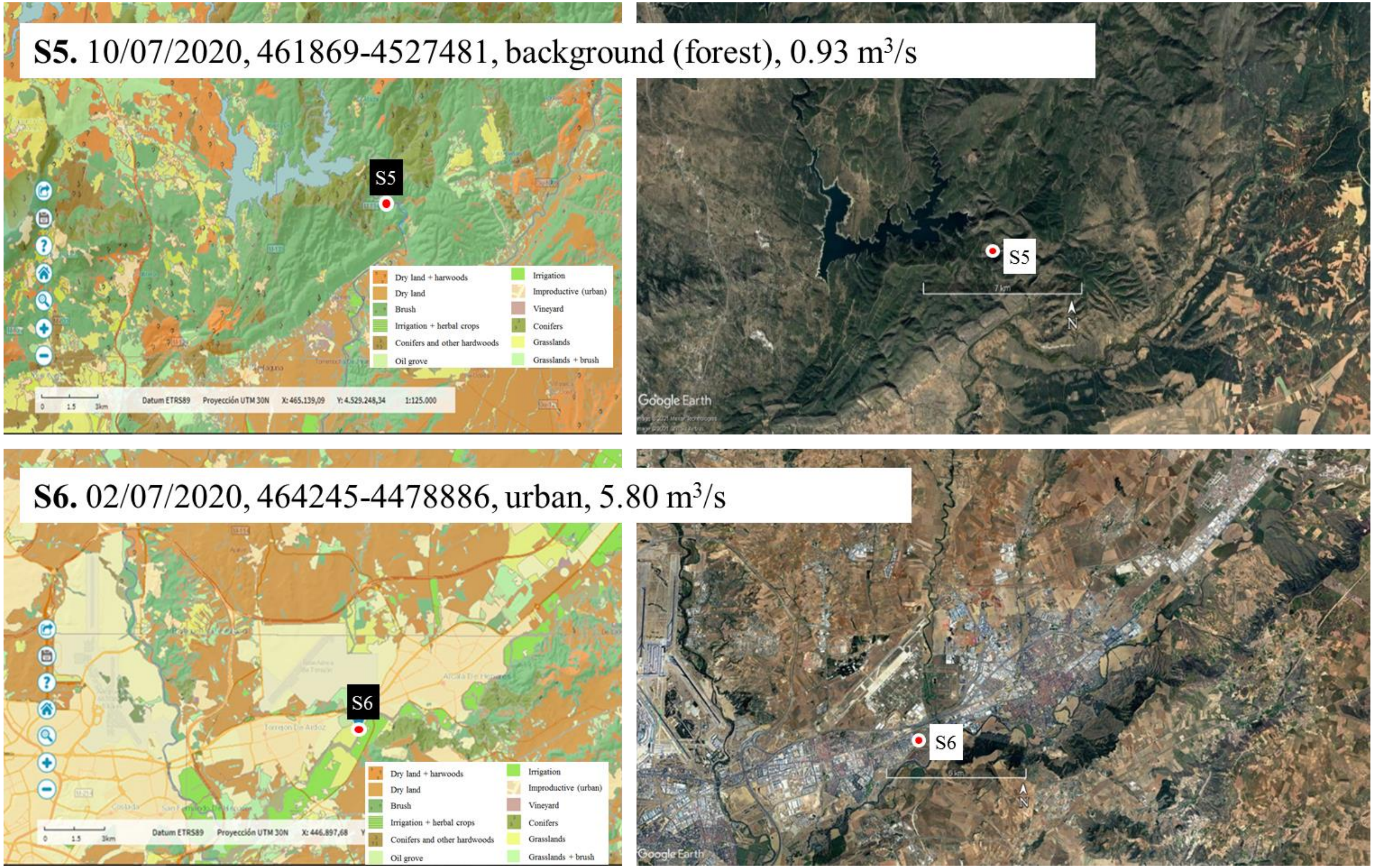


Figure S1 Continuation. Sample, date, location (UTMX-UTMY), soil characterization and water flow (m^3/s) at each sampling site.

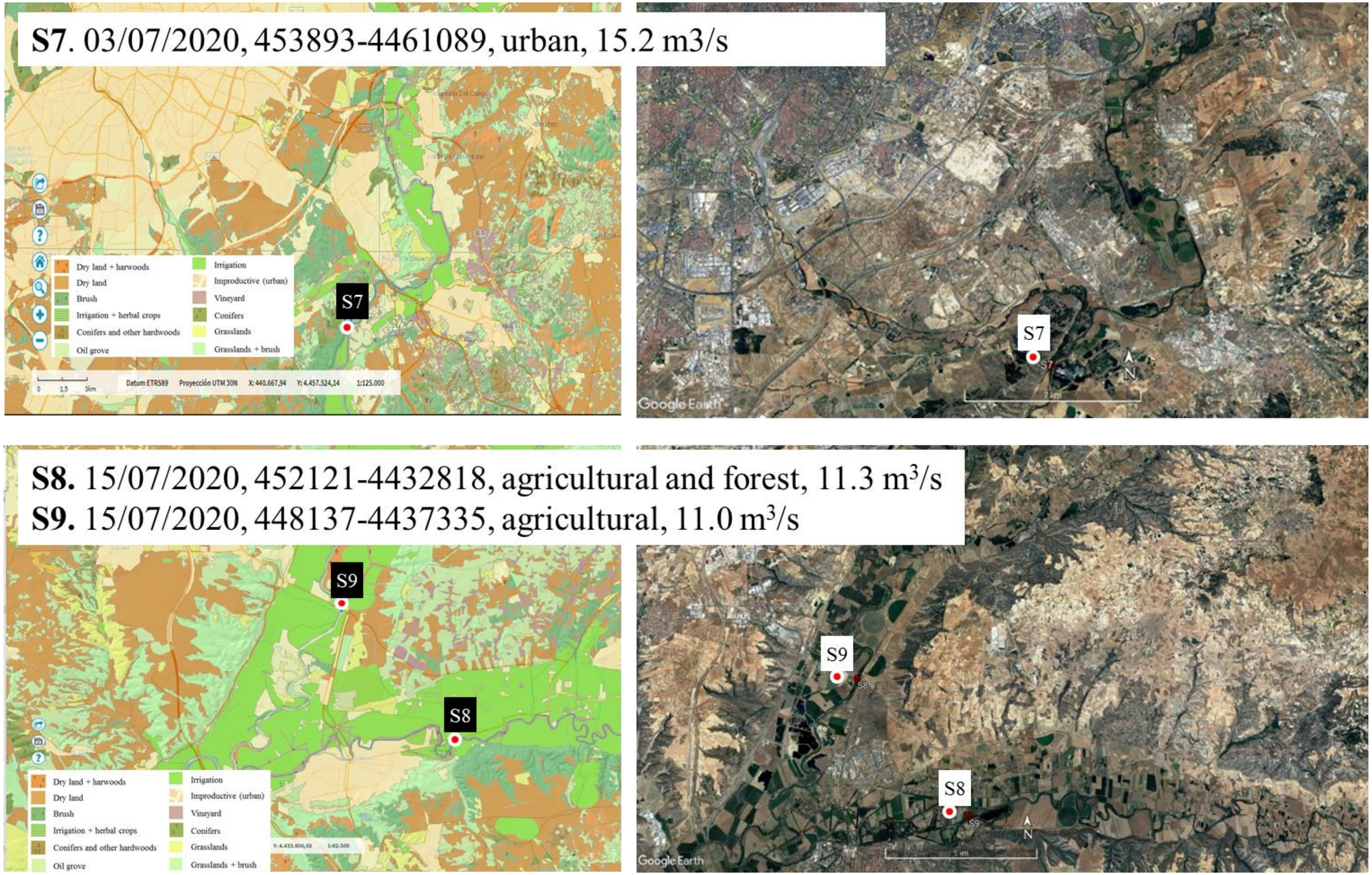


Figure S1 Continuation. Sample, date, location (UTMX-UTMY), soil characterization and water flow (m^3/s) at each sampling site.

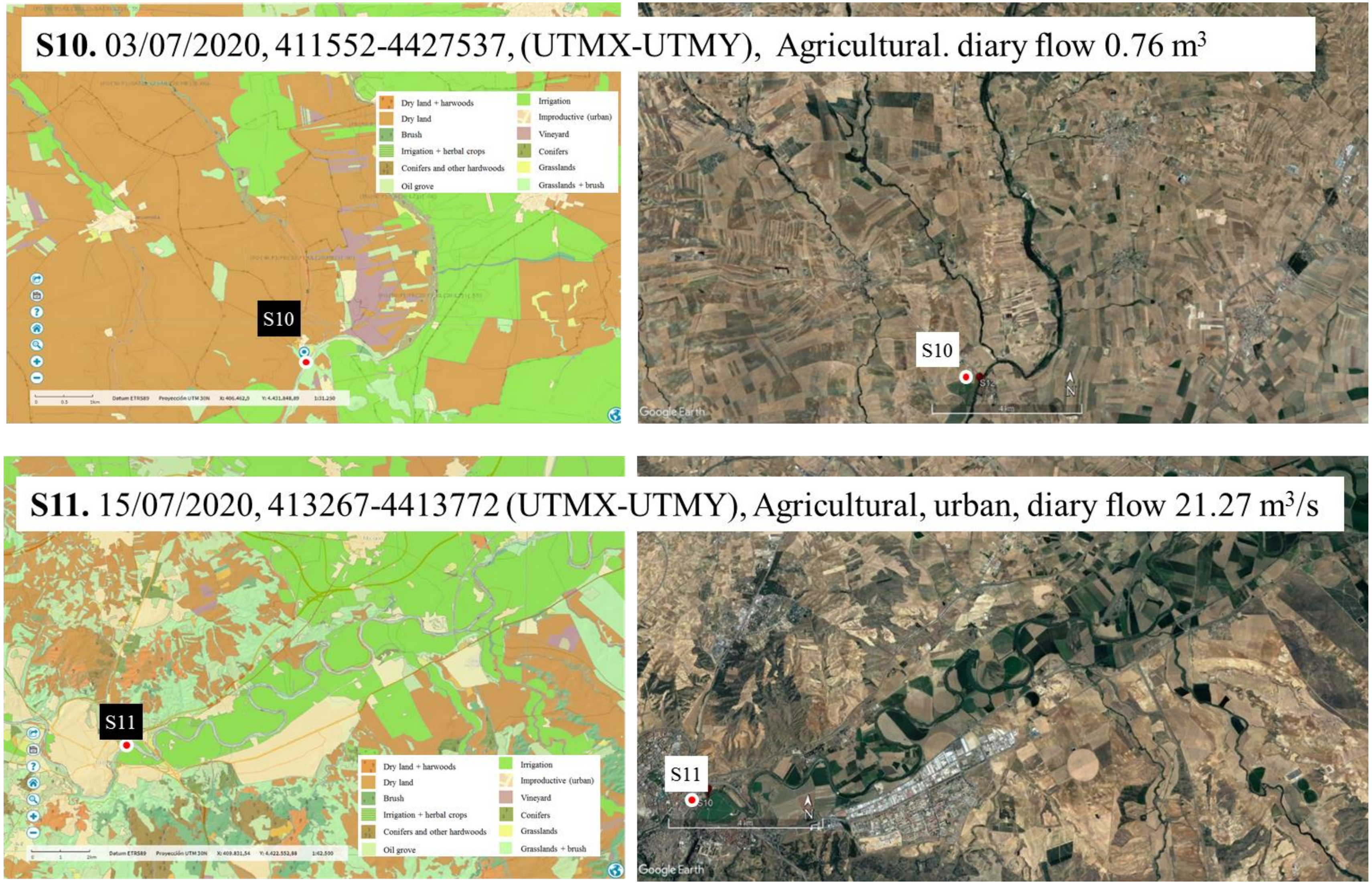


Figure S1 Continuation. Sample, date, location (UTMX-UTMY), soil characterization and water flow (m^3/s) at each sampling site.

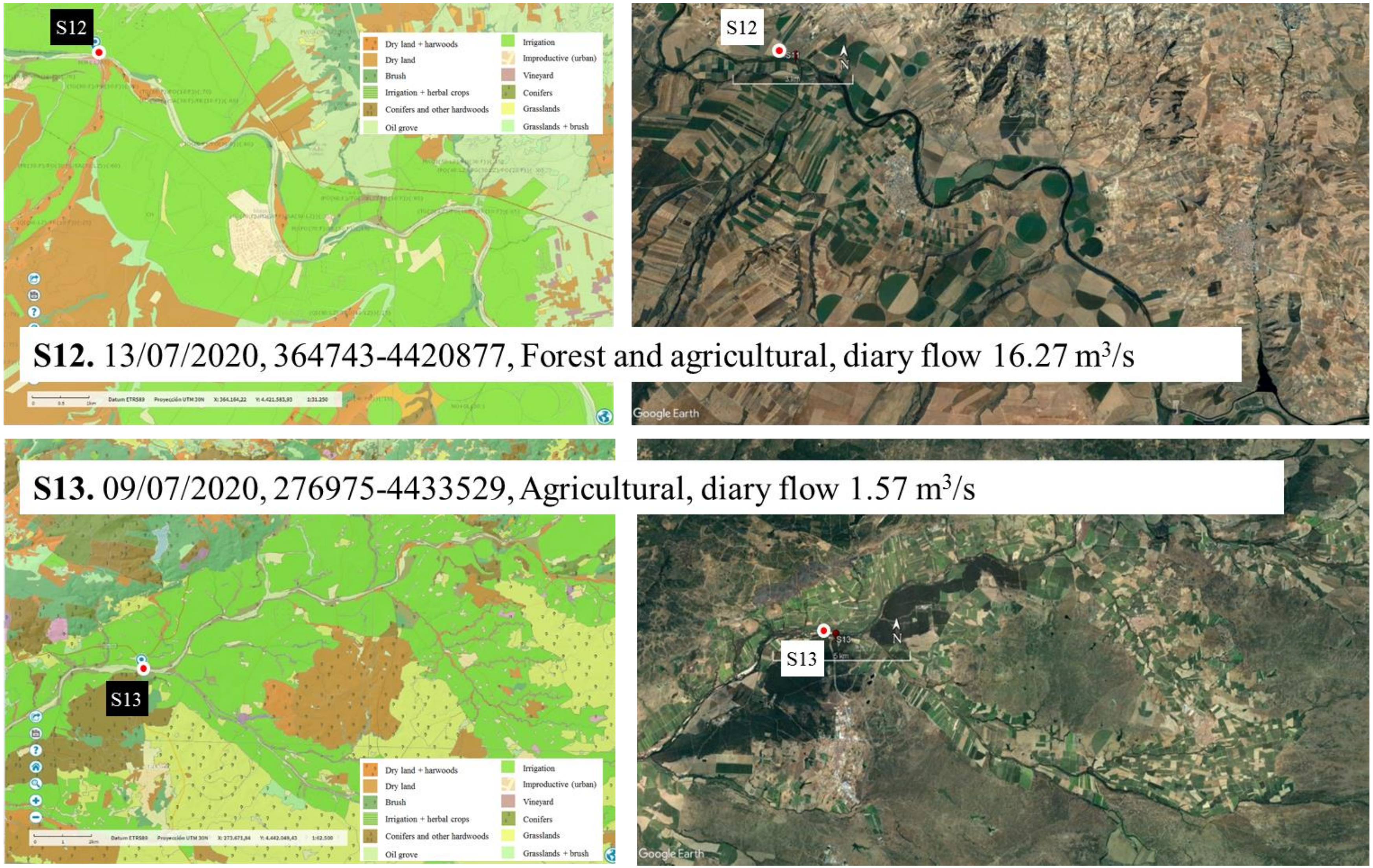


Figure S1 Continuation. Sample, date, location (UTMX-UTMY), soil characterization and water flow (m^3/s) at each sampling site.

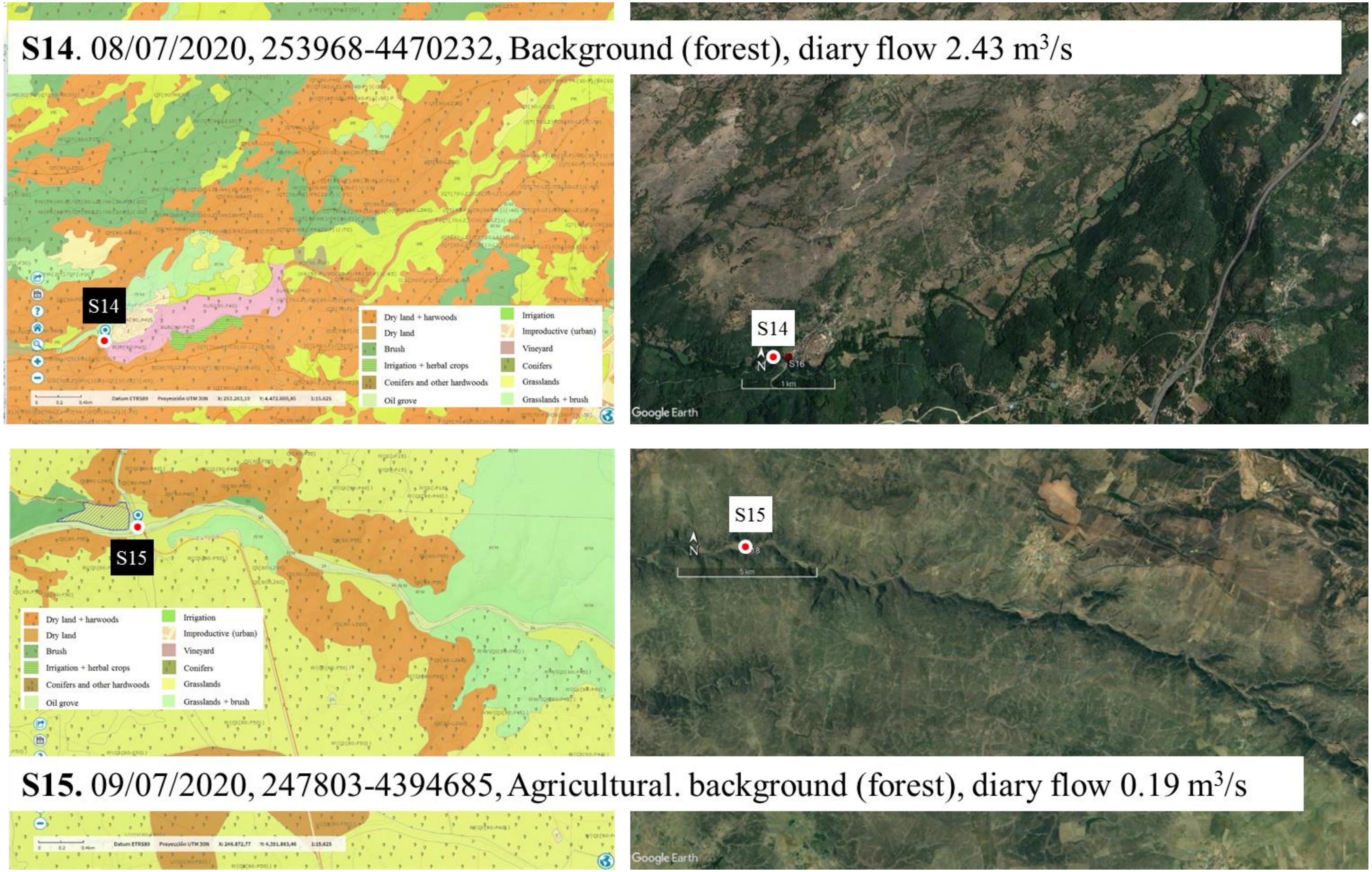


Figure S1 Continuation. Sample, date, location (UTMX-UTMY), soil characterization and water flow (m^3/s) at each sampling site.

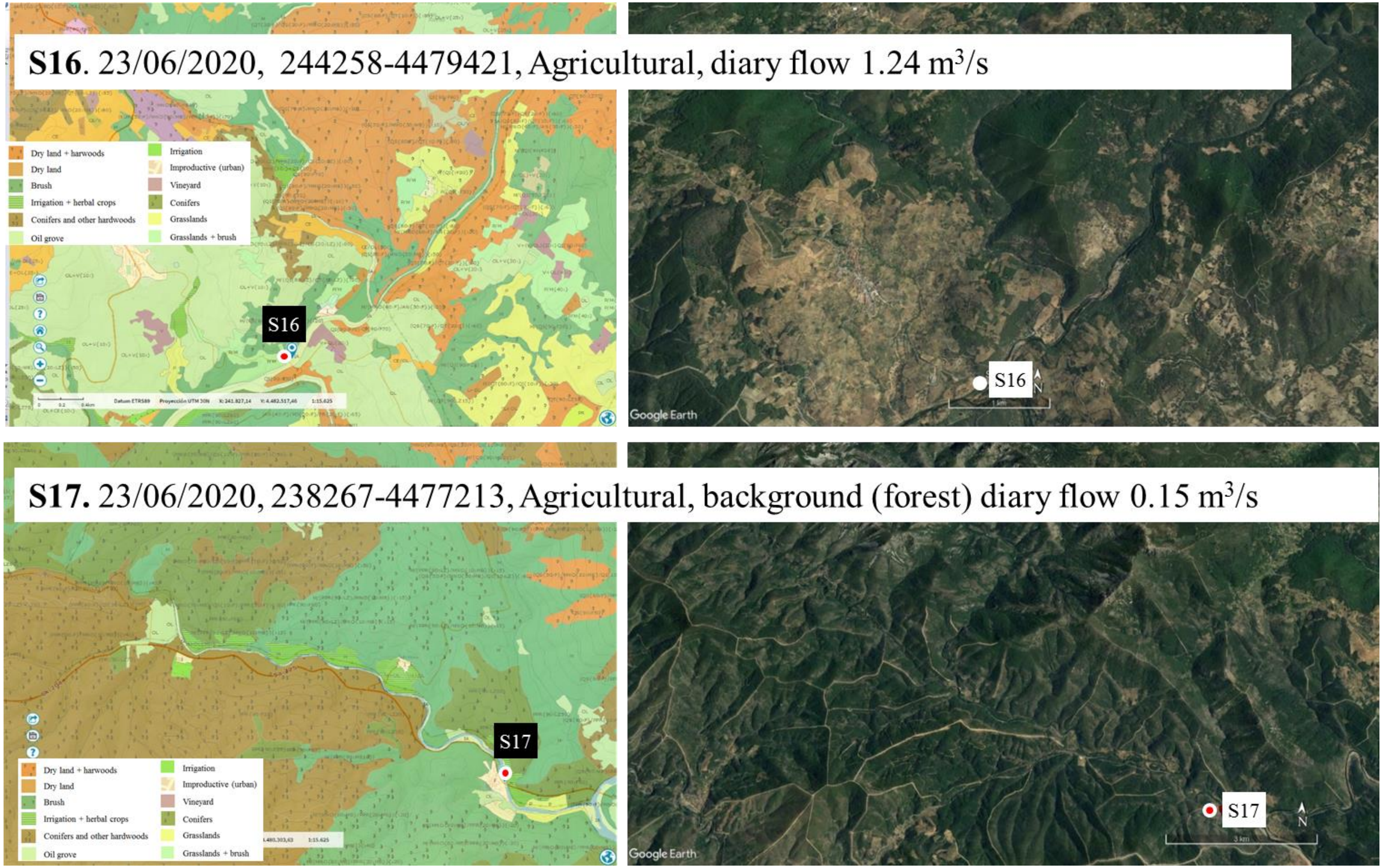


Figure S1 Continuation. Sample, date, location (UTMX-UTMY), soil characterization and water flow (m^3/s) at each sampling site

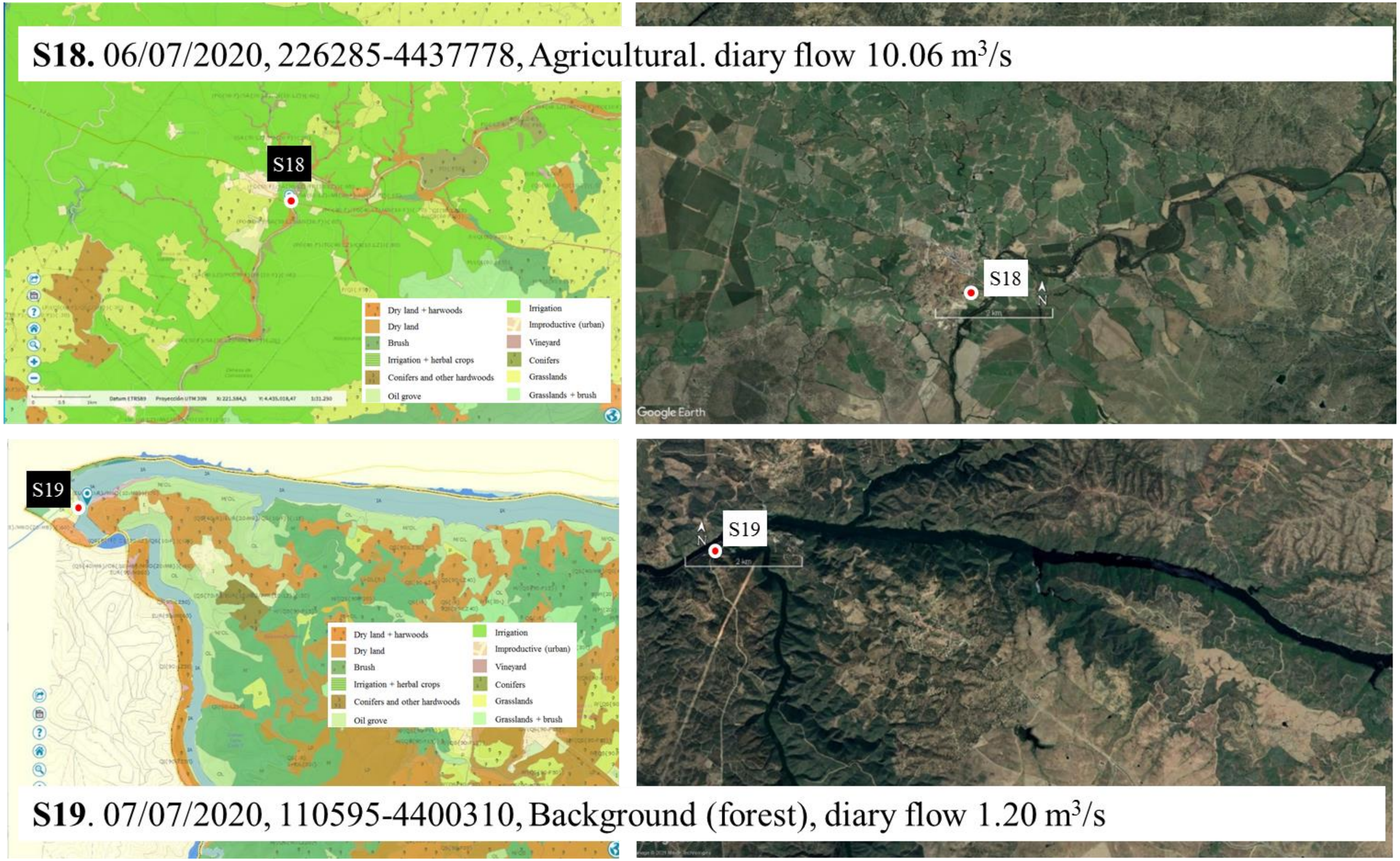
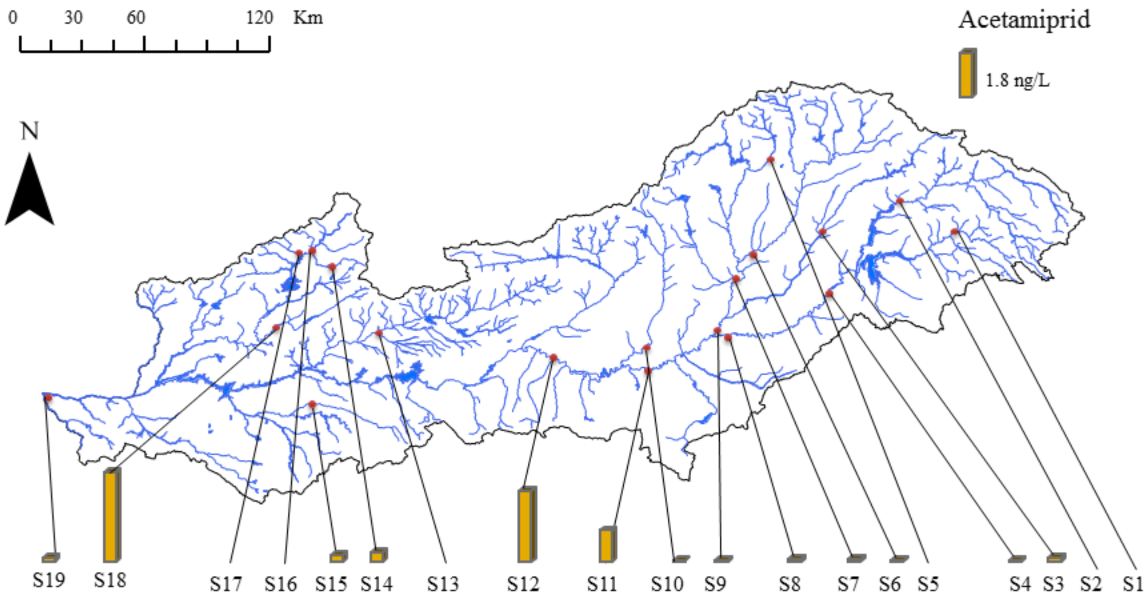
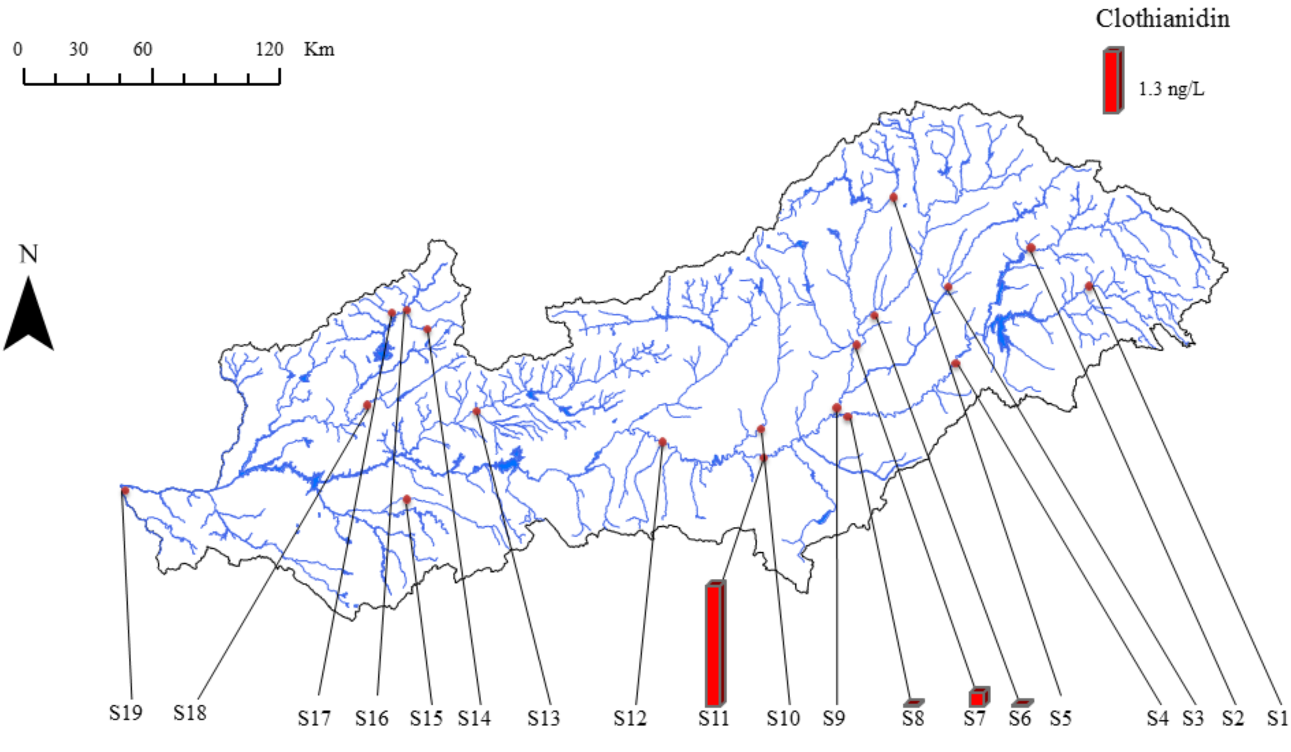
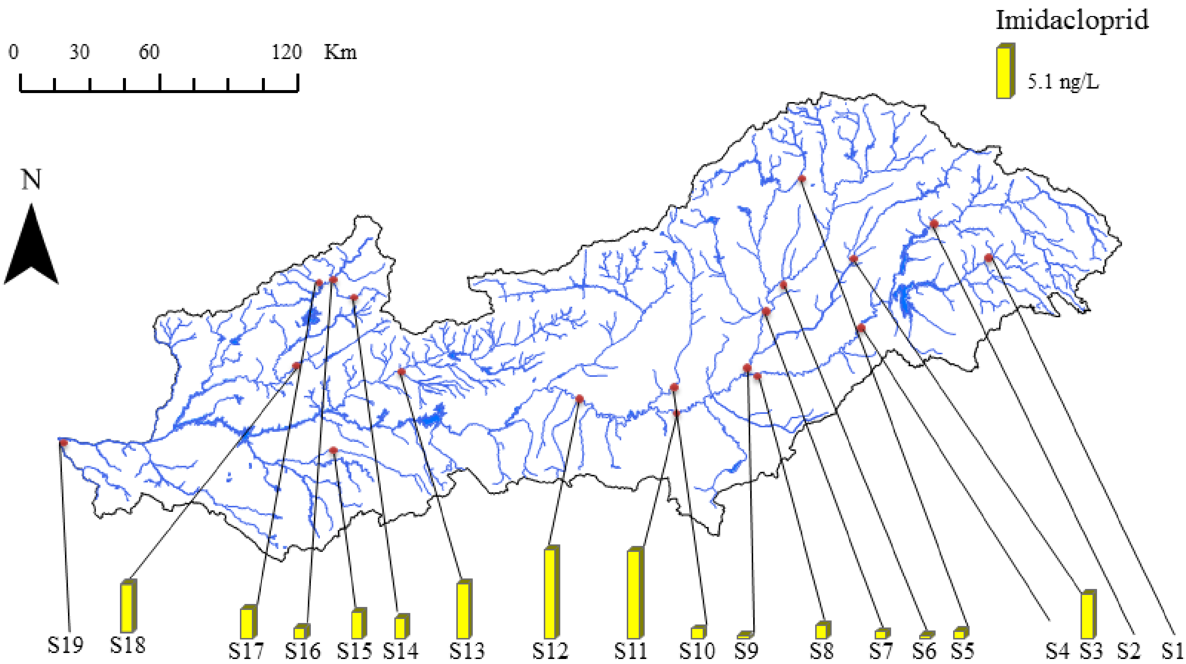
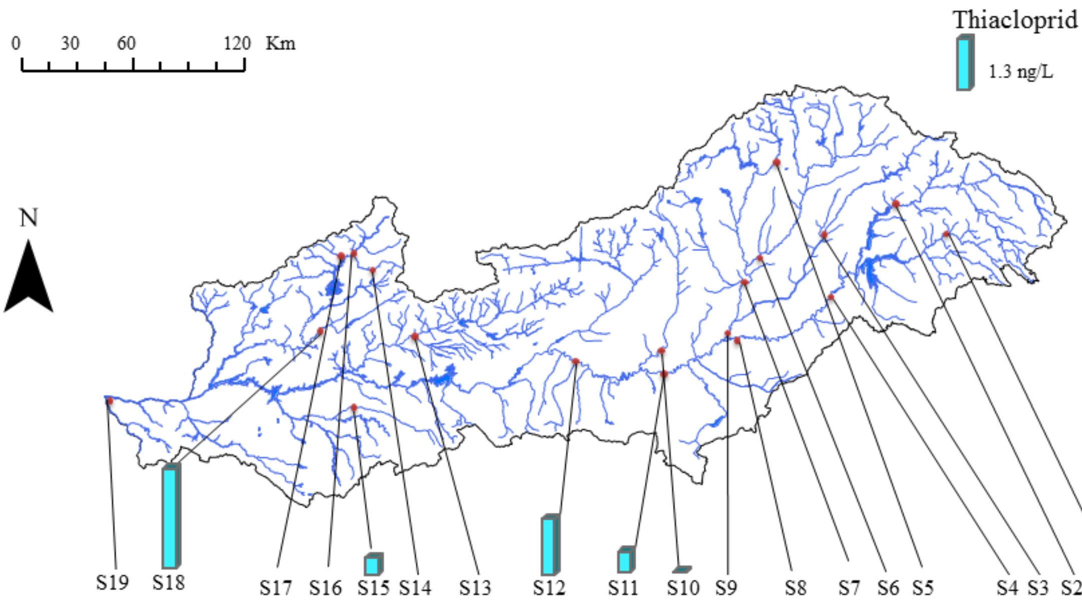


Figure S1 Continuation. Sample, date, location (UTMX-UTMY), soil characterization and water flow (m^3/s) at each sampling site.


0 30 60 120 Km
N
Acetamiprid
1.8 ng/L
S19 S18 S17 S16 S15 S14 S13 S12 S11 S10 S9 S8 S7 S6 S5 S4 S3 S2 S1


0 30 60 120 Km
N
Clothianidin
1.3 ng/L
S19 S18 S17 S16 S15 S14 S13 S12 S11 S10 S9 S8 S7 S6 S5 S4 S3 S2 S1


Imidacloprid
5.1 ng/L
0 30 60 120 Km
N
S19
S18
S17
S16
S15
S14
S13
S12
S11
S10
S9
S8
S7
S6
S5
S4
S3
S2
S1


Thiacloprid
1.3 ng/L
0 30 60 120 Km
N
S19
S18
S17
S16
S15
S14
S13
S12
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S10
S9
S8
S7
S6
S5
S4
S3
S2
S1

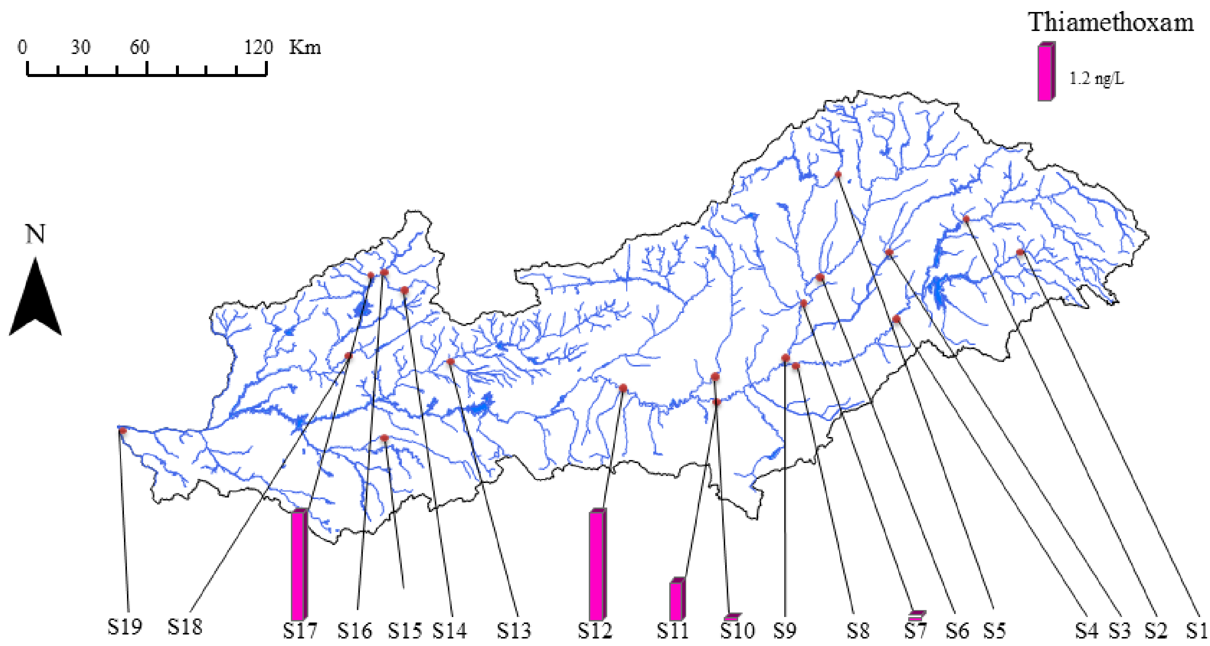

Figure S2. Concentrations (ng/L) of acetamiprid, imidacloprid, chlotianidin, thiamethoxam and thiacloprid in surface water.

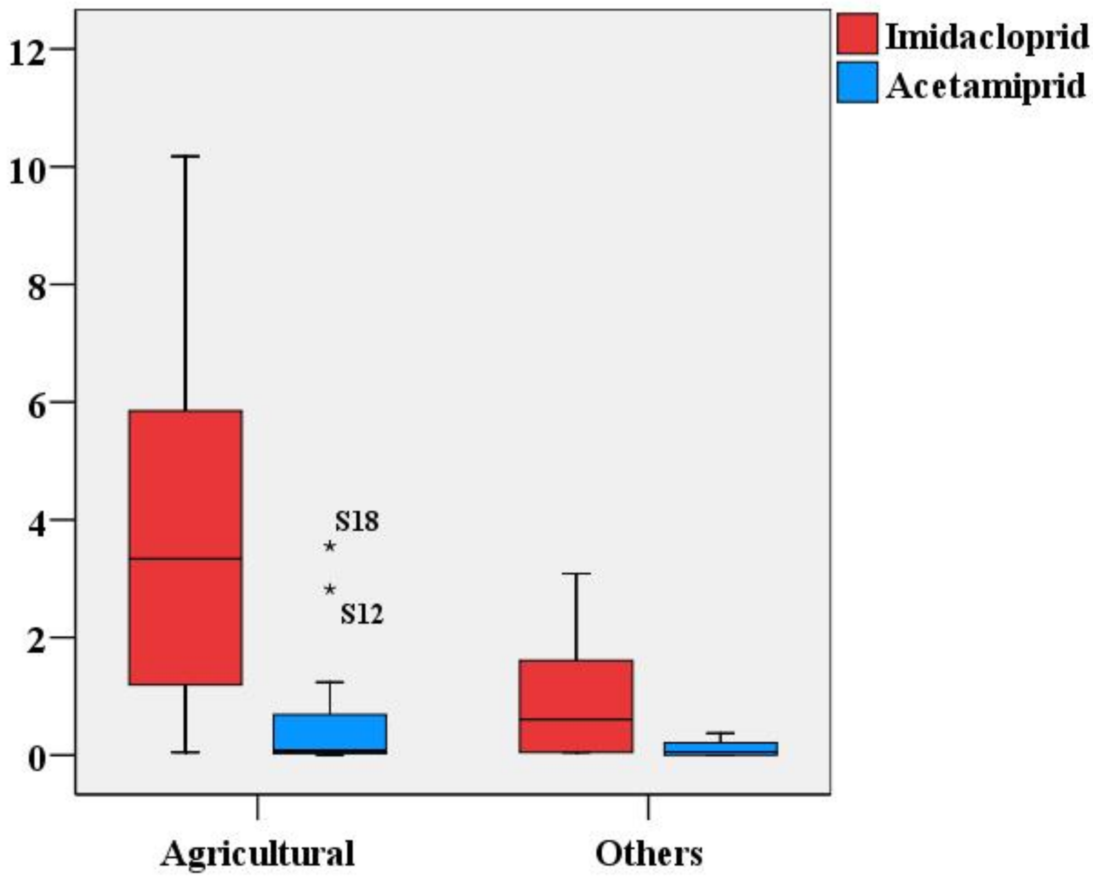

Figure S3. Box and whisker diagram of imidacloprid and acetamiprid concentrations (ng/L) in surface water.

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