Agricultural Water Management

Multiple environmental benefits of alternate wetting and drying irrigation system with limited yield impact on European rice cultivation: the Ebre Delta case

--Manuscript Draft--

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Abstract:	The alternate wetting and drying (AWD) is an irrigation technology for rice cultivation, consisting in implementing alternate draining and flooded periods over the growing season, that delivers multiple environmental benefits, such as reduced water consumption, CH_4 emissions and arsenic (As) grain content, but can be offset by yield losses. The trade-offs between the agronomic and environmental effects of AWD are crop context-dependent and they also vary among the different versions of AWD studied. Therefore, the implementation of a safe AWD needs to be preceded by studies conducted within a specific rice cropping system. A two-year field experiment was conducted to assess the effect of AWD on grain yield, As and heavy metal content in grains, and greenhouse gas emissions in nine representative European rice cultivars grown in a Mediterranean growing area. The experiment was performed in a split-plot design with four replications. The study revealed a significant cultivar effect on the agronomic response to AWD. Among the studied cultivars, one of them performed as tolerant to AWD while a group formed by four cultivars showed slight non-significant yield decline. AWD significantly reduced CH_4 emissions and the global warming potential by 90% being such a large mitigation capacity explained by the negligible N_2O emissions found in both water treatments. Finally, the implementation of AWD significantly reduced by *ca.* 40 % As grain concentration but increased cadmium content, though the levels remained below the recommended thresholds. Further, AWD-induced increases in key nutritional elements like cupper, selenium and zinc were also found. In conclusion, this study confirms that AWD can be safely implemented in Mediterranean rice cultivation conditions with limited or null yield impact while obtaining the associated environmental benefits of this practice.
Suggested Reviewers:	Benjamin Runkle, PhD Researcher, Arkansas State University brrunkle@uark.edu Dr. Runkle is an expert rice research and he has studied the effect of AWD on greenhouse gas emissions.
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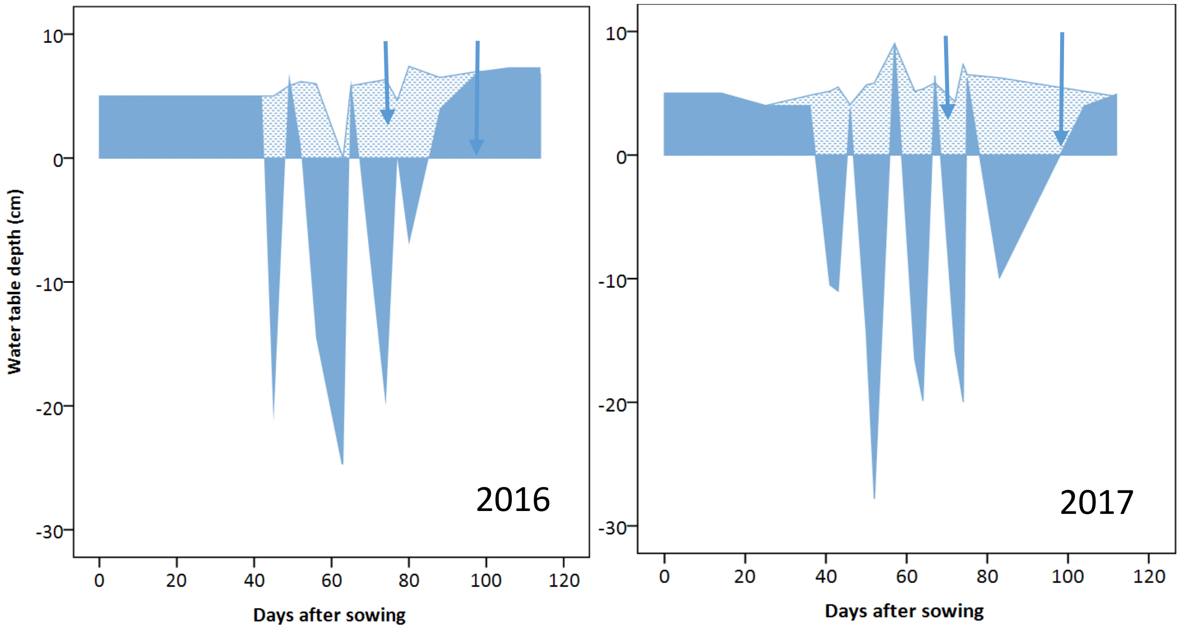
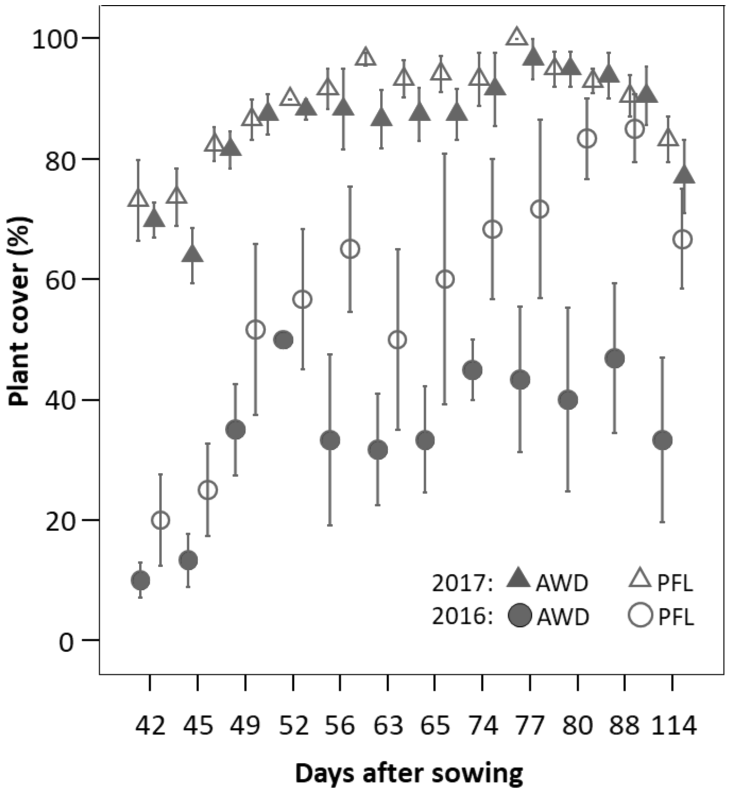
	Researcher, University of California Davis drcarrijo@ucdavis.edu Dr. Carrijo is an expert in the implementation of AWD on rice cultivation . Her meta-analyses on AWD implementation is very well recognized
	Gareth J. Norton, PhD Researcher, University of Aberdeen g.norton@abdn.ac.uk Dr. Norton has an extensive research on AWD implementation in rice cultivation in Bangladesh, with special focus on element (arsenic and heavy metals) concentration in rice.
Opposed Reviewers:	
Response to Reviewers:	

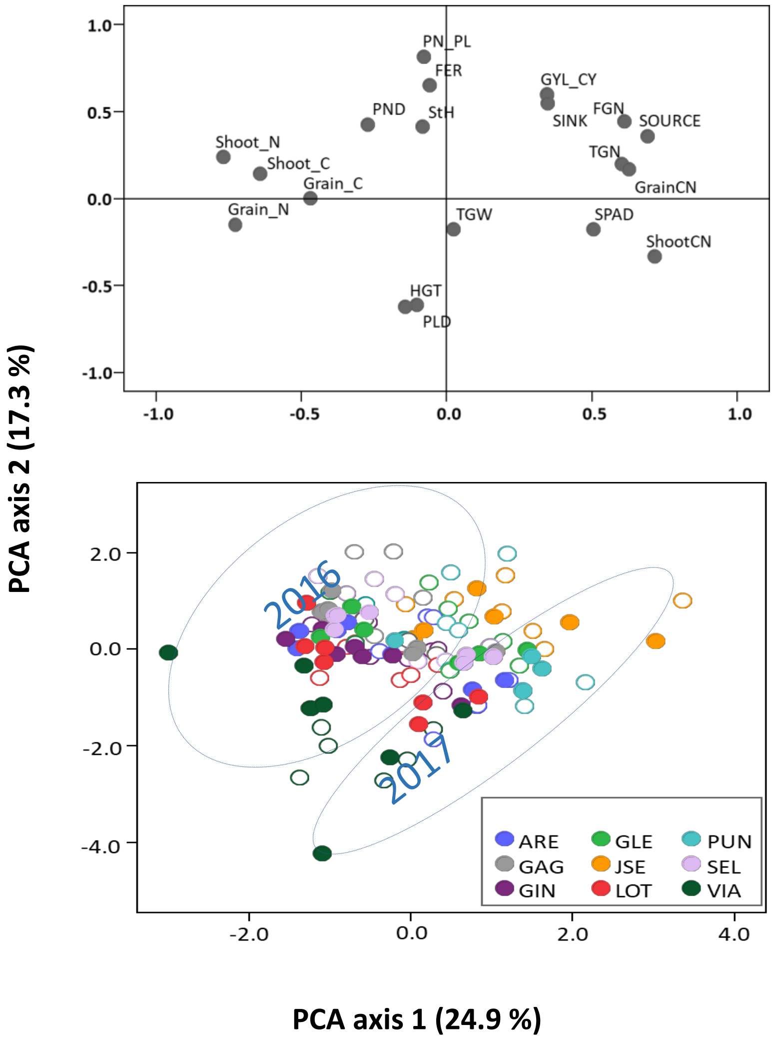
ABSTRACT

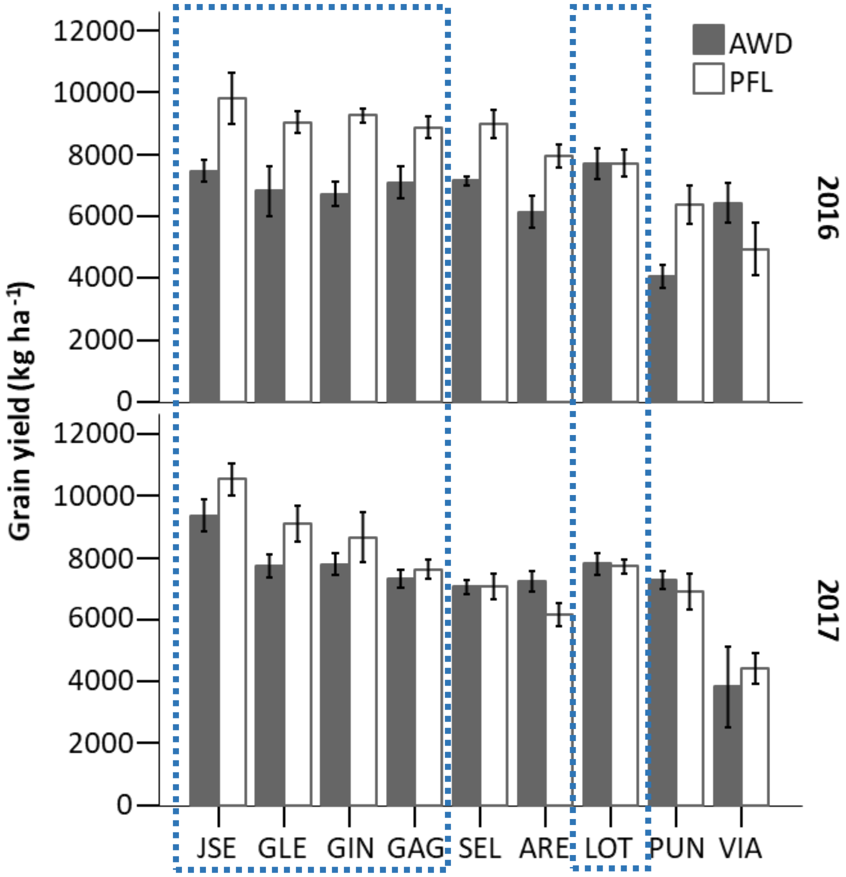
The AWD is an irrigation technology for rice cultivation, consisting in implementing alternate draining and flooded periods over the growing season, that delivers multiple environmental benefits, such as reduced water consumption, CH_4 emissions and arsenic (As) grain content, but can be offset by yield losses. The trade-offs between the agronomic and environmental effects of AWD are crop context-dependent and they also vary among the different versions of AWD studied. Therefore, the implementation of a safe AWD needs to be preceded by studies conducted within a specific rice cropping system. A two-year field experiment was conducted to assess the effect of AWD on grain yield, As and heavy metal content in grains, and greenhouse gas emissions in nine representative European rice cultivars grown in a Mediterranean growing area. The experiment was performed in a split-plot design with four replications. The study revealed a significant cultivar effect on the agronomic response to AWD. Among the studied cultivars, one of them performed as tolerant to AWD while a group formed by four cultivars showed slight non-significant yield decline. AWD significantly reduced CH_4 emissions and the global warming potential by 90% being such a large mitigation capacity explained by the negligible N_2O emissions found in both water treatments. Finally, the implementation of AWD significantly reduced by *ca.* 40 % As grain concentration but increased cadmium content, though the levels remained below the recommended thresholds. Further, AWD increased key nutritional elements like cupper, selenium, and zinc. In conclusion, this study confirms that AWD can be safely implemented in Mediterranean rice cultivation conditions with limited or null yield impact while obtaining the associated environmental benefits of this practice.

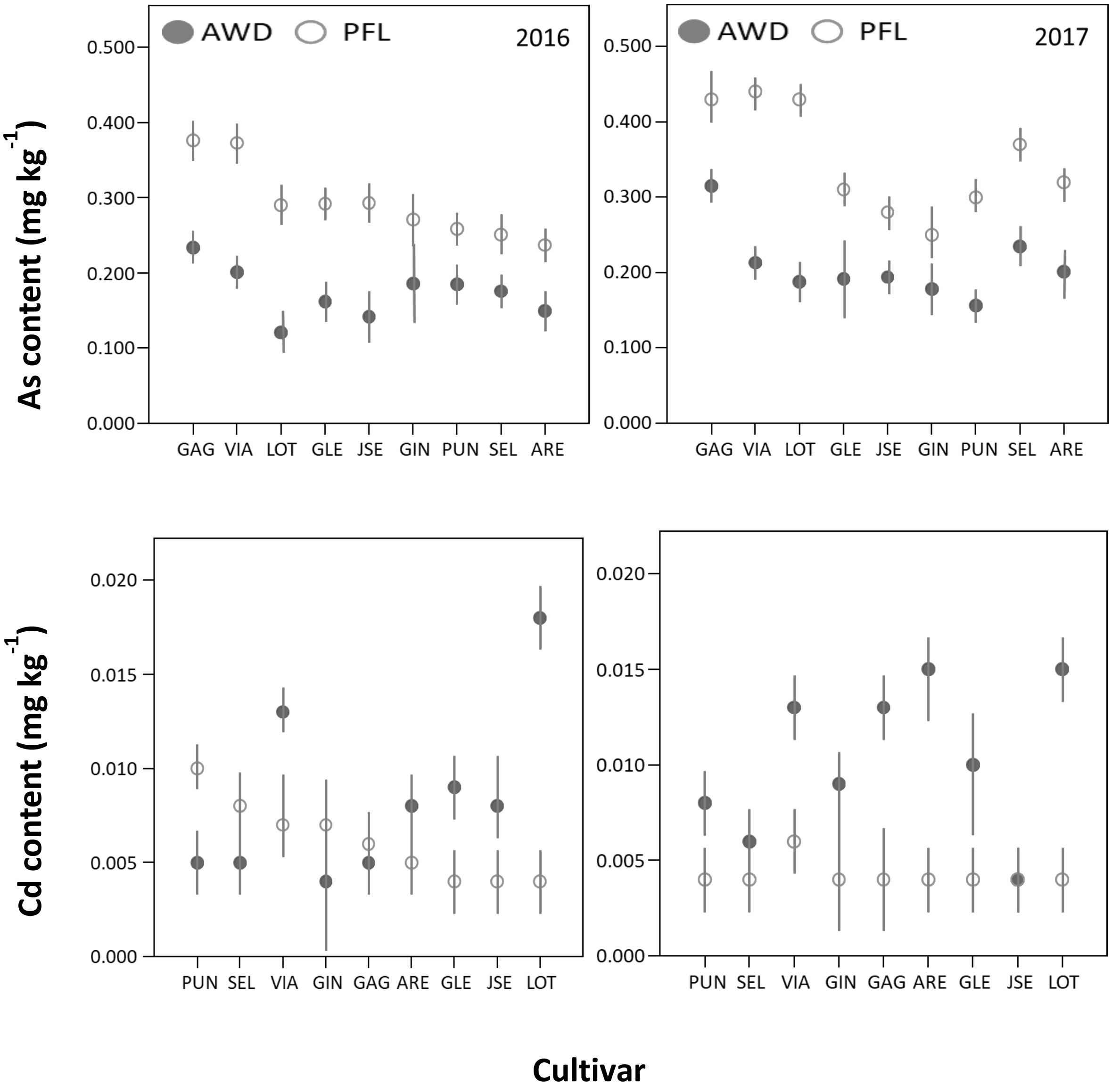
Keywords: Alternate wetting and drying, greenhouse gas emission, global warming potential, heavy metal, rice production

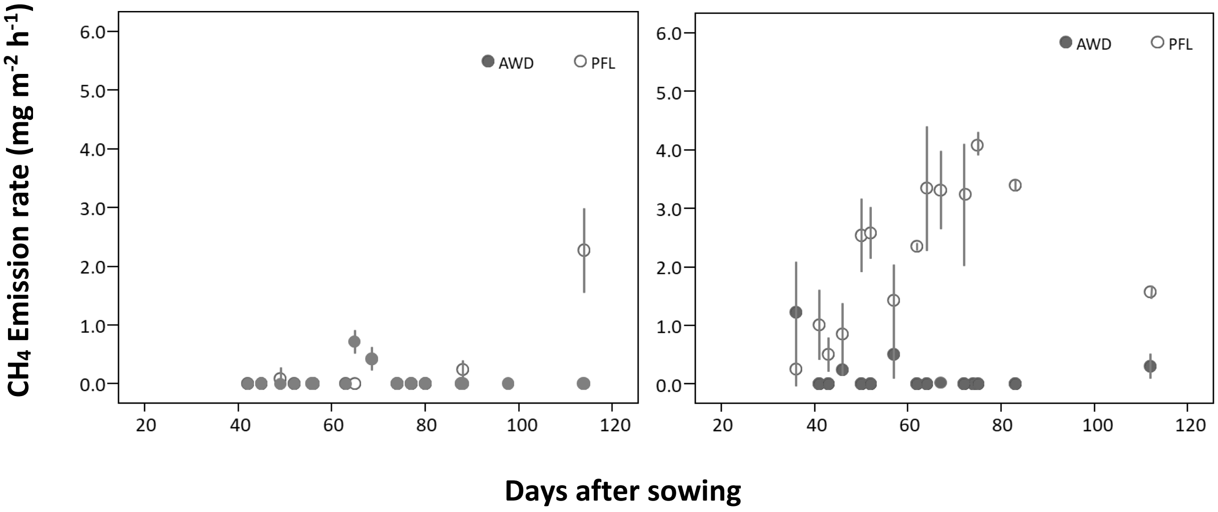

Water table depth (cm)
10
0
-10
-20
-30
0
20
40
60
80
100
120
Days after sowing
2016
2017

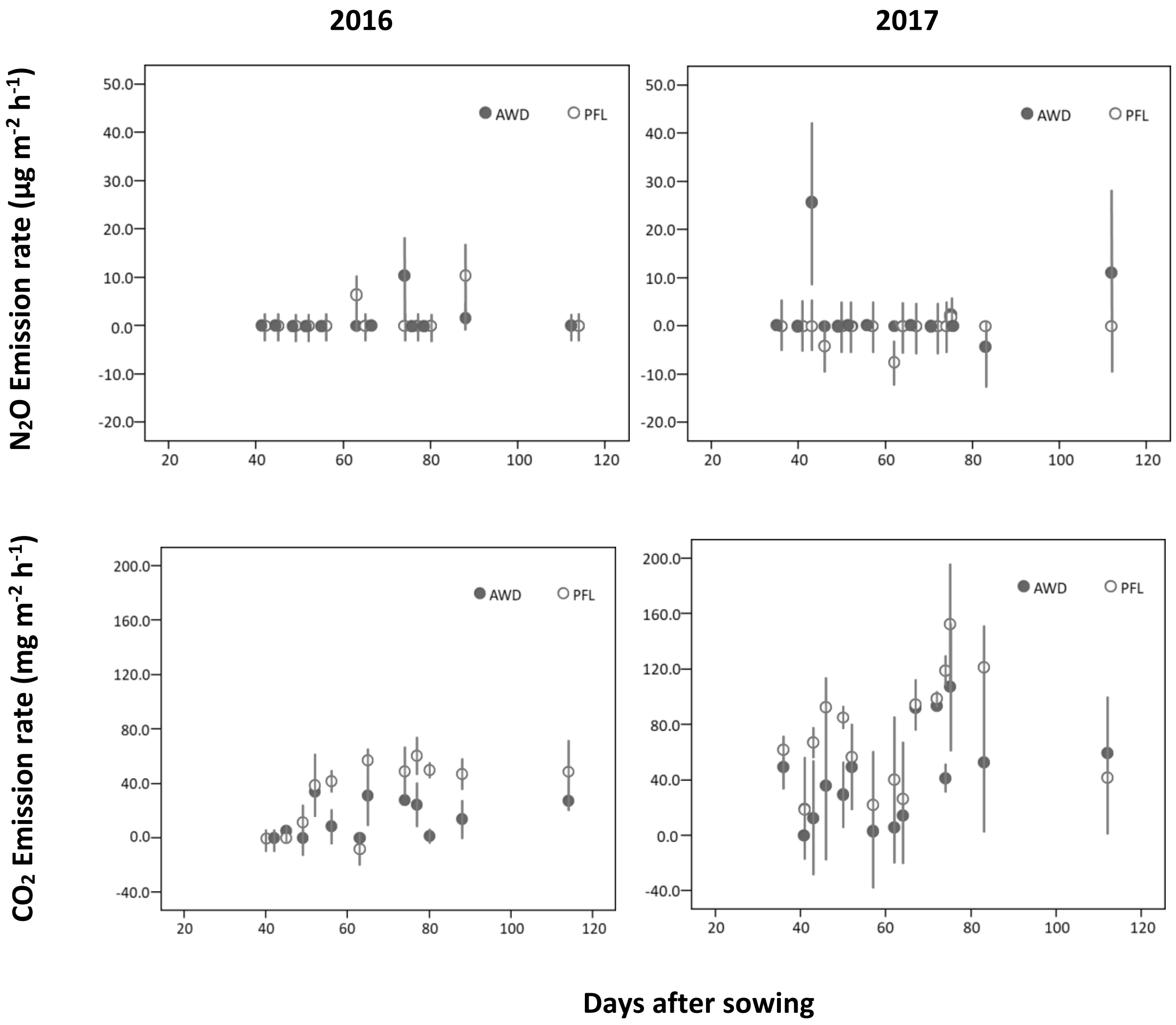

Plant cover (%)
100
80
60
40
20
0
42
45
49
52
56
63
65
74
77
80
88
114
Days after sowing
2017: ▲ AWD △ PFL
2016: ● AWD ○ PFL


PN_PL
FER
PND
StH
GYL_CY
SINK
FGN
SOURCE
Shoot_N
Shoot_C
Grain_C
TGN
GrainCN
Grain_N
TGW
SPAD
ShootCN
HGT
PLD
1.0
0.5
0.0
-0.5
-1.0
-1.0
-0.5
0.0
0.5
1.0
2016
2017
2.0
0.0
-2.0
-4.0
-2.0
0.0
2.0
4.0
● ARE
● GAG
● GIN
● GLE
● JSE
● LOT
● PUN
● SEL
● VIA
PCA axis 2 (17.3 %)
PCA axis 1 (24.9 %)


Grain yield (kg ha⁻¹)
12000
10000
8000
6000
4000
2000
0
AWD
PFL
2016
2017
JSE
GLE
GIN
GAG
SEL
ARE
LOT
PUN
VIA


● AWD ○ PFL
2016
2017
As content (mg kg^{-1})
0.000
0.100
0.200
0.300
0.400
0.500
GAG VIA LOT GLE JSE GIN PUN SEL ARE
Cd content (mg kg^{-1})
0.000
0.005
0.010
0.015
0.020
PUN SEL VIA GIN GAG ARE GLE JSE LOT
Cultivar


CH₄ Emission rate (mg m⁻² h⁻¹)
6.0
5.0
4.0
3.0
2.0
1.0
0.0
AWD
PFL
20
40
60
80
100
120
Days after sowing


2016
2017
N_2O Emission rate (µg m^{-2} h^{-1})
● AWD
○ PFL
50.0
40.0
30.0
20.0
10.0
0.0
-10.0
-20.0
CO_2 Emission rate (mg m^{-2} h^{-1})
200.0
160.0
120.0
80.0
40.0
0.0
-40.0
20
40
60
80
100
120
Days after sowing

Crop practice		**2016**	**2017**
Sowing (500 viable seeds m^{-2})		2/5/2016	4/5/2017
Fertilization	Basal (120 Kg N ha^{-1}; 27-8-10)	19/4/2016	21/4/2017
	Topdressing (50 Kg N ha^{-1}, ammonium sulphate)	5/7/2016	7/7/2017
Herbicides	Pre-emergence (Oxadiazon 38 % p/V)	25/4/2016	26/4/2017
	Echinochloa spp. and Cyperaceae (Penoxulam 2.04 % p/v; Halosulfuron-metil 75 % p/p)	3/6/2016	29/5/2017
	Ciperaceae and alismataceae (Penoxulam 2.04 % p/v; Bentazona 87 % p/v; sal amina 60 % p/v)	30/6/16	26/6/17
Fungicides	Triciclazol 75 % p/v, Tebuconazol 25 % p/v	2/8/16	
	Tebuconazol 25 %, p/v		18/7/17
	Pixoxistrobin 25 % p/v		31/7/17
	Azoxistrobyn 25 % p/v	16/8/16	16/8/17
Harvest		22/9/2016 – 4/10/2016	20/9/201– 6/10/2017

Phenological stage	2016		2017	
	Water table depth (cm)	Soil water status (KPa)	Water table depth (cm)	Soil water status (KPa)
Vegetative stage	-21 ± 1	-10 ± 5	-11 ± 4	- 15 ± 3
			-28 ± 6	-33 ± 9
Early reproductive stages	- 25 ± 0	- 53 ± 14	-20 ± 2	-19 ± 3
Booting or jointing	-20 ± 1	-10 ± 4	-20 ± 2	-45 ± 11
Prior to flowering	- 7 ± 5	0 ± 0	-13 ± 3	-16 ± 4

			AWD						PFL					
			GYL	PND	TGN	TGW	FER	SINK	GYL	PND	TGN	TGW	FER	SINK
2016	Cultivars	**ARE**	6146 ± 516	375 ± 47	64 ± 5	28.3 ± 0.2	93.0 ± 1.2	23499 ± 2418	7951 ± 372	433 ± 8	78 ± 7	28.8 ± 1.1	83.5 ± 2.7	33702 ± 3769
		GAG	7077 ± 516	511 ± 22	55 ± 2	26.1 ± 0.2	97.1 ± 0.2	28017 ± 2195	8872 ± 356	508 ± 23	74 ± 1	25.5 ± 0.2	93.1 ± 0.5	37393 ± 1479
		GIN	6723 ± 375	455 ± 43	49 ± 3	31.9 ± 0.1	95.7 ± 0.3	22021 ± 1256	9253 ± 240	487 ± 9	62 ± 2	33 ± 0.4	92.8 ± 0.6	30214 ± 644
		GLE	6814 ± 799	389 ± 44	52 ± 1	35.7 ± 0.7	94.5 ± 0.5	20080 ± 2077	9042 ± 361	406 ± 20	68 ± 4	36.4 ± 0.3	90.5 ± 0.7	27501 ± 1309
		JSE	7473 ± 345	318 ± 37	67 ± 8	36.8 ± 0.3	97.2 ± 0.3	20915 ± 1010	9822 ± 818	361 ± 22	77 ± 5	36.8 ± 0.4	96.6 ± 0.1	27645 ± 2228
		LOT	7682 ± 496	508 ± 40	49 ± 1	32.5 ± 0.4	94.5 ± 0.6	25093 ± 2033	7705 ± 427	422 ± 16	68 ± 2	31.2 ± 0.3	86.3 ± 1.3	28606 ± 1406
		PUN	4042 ± 375	202 ± 14	92 ± 3	23.7 ± 0	91 ± 1.2	18779 ± 1884	6376 ± 616	295 ± 27	123 ± 10	22.6 ± 0.3	78.5 ± 2.6	35775 ± 2809
		SEL	7142 ± 158	475 ± 14	60 ± 1	26.4 ± 0.2	95.7 ± 0.2	28304 ± 639	8983 ± 449	513 ± 30	73 ± 3	26.4 ± 0.3	91.9 ± 1.1	37139 ± 2186
		VIA	6433 ± 644	393 ± 39	46 ± 2	38.2 ± 0.5	94.6 ± 0.5	17889 ± 1969	4946 ± 829	283 ± 23	62 ± 4	34 ± 0.9	80.4 ± 2.8	17703 ± 2072
2017	Cultivars	**ARE**	7243 ± 327	400 ± 30	79 ± 8	27.2 ± 1	85.8 ± 1.2	31267 ± 2019	6174 ± 382	397 ± 7	68 ± 3	28 ± 0.8	82.1 ± 1.8	26821 ± 1220
		GAG	7317 ± 280	483 ± 13	62 ± 1	26.3 ± 0.2	93.1 ± 0.4	29869 ± 1075	7628 ± 292	492 ± 14	63 ± 2	27.3 ± 0.3	90.4 ± 1	30889 ± 888
		GIN	7787 ± 355	442 ± 5	61 ± 1	33.7 ± 0.6	85.3 ± 3.5	27097 ± 391	8660 ± 800	460 ± 33	67 ± 4	33.1 ± 0.8	83.3 ± 3.9	30798 ± 2414
		GLE	7740 ± 379	376 ± 11	73 ± 1	34.6 ± 0.4	82.3 ± 3.9	27260 ± 1115	9099 ± 587	393 ± 21	75 ± 2	35.9 ± 0.6	86.3 ± 1.8	29455 ± 1986
		JSE	9353 ± 522	371 ± 21	74 ± 2	36.5 ± 0.2	93.9 ± 0.5	27322 ± 1471	10526 ± 502	392 ± 4	78 ± 3	37.2 ± 0.6	92.7 ± 0.4	30559 ± 1312
		LOT	7803 ± 352	417 ± 18	66 ± 2	32.1 ± 0.3	88.5 ± 0.2	27480 ± 1433	7720 ± 221	416 ± 13	69 ± 1	32.7 ± 0.3	82.7 ± 1.2	28642 ± 1257
		PUN	7284 ± 299	331 ± 20	105 ± 5	25 ± 0.8	85.1 ± 2.5	34376 ± 1693	6896 ± 564	312 ± 24	110 ± 6	24.6 ± 0.7	82.6 ± 2.4	34201 ± 3152
		SEL	7056 ± 244	385 ± 24	78 ± 1.2	26.4 ± 0.3	87.5 ± 2.6	29829 ± 1629	7065 ± 413	445 ± 21	71 ± 3	26.2 ± 0.5	85.9 ± 2.3	31277 ± 825
		VIA	3834 ± 1305	267 ± 29	67 ± 4.2	33.8 ± 2.2	54.8 ± 10	18093 ± 2999	4430 ± 504	264 ± 14	68 ± 3	35.8 ± 1	69.1 ± 5.3	17829 ± 770

	Year	**WM**	**CV**	**YxWM**	**YxCV**	**WMxCV**	**YxWMxCV**
	$F_{1,107}$	$F_{1,107}$	$F_{8,107}$	$F_{1,107}$	$F_{8,107}$	$F_{8,107}$	$F_{8,107}$
GYL_CY	0.13	26.47***	23.22***	11.41***	4.19***	2.34*	2.05*
PND	3.91	1.47	34.8***	0.01	3.99***	2.18*	1.84
TGN	23.77***	43.58***	57.06***	39.51***	2.09*	1.6	0.81
TGW	0.34	0.01	218.34***	6.73*	2.4*	1.08	2.77**
FER	77.09***	15.51***	20.93***	14.23***	8.07***	1.34	3.04**
SINK	8.55**	49.61***	20.42***	31.79***	2.93**	1.74	2.3*

	Y	**WM**	**CV**	**Y×WM**	**Y×CV**	**WM×CV**	**Y×WM×CV**
	$F_{1, 25}$	$F_{1, 25}$	$F_{1, 25}$	$F_{1, 25}$	$F_{7, 25}$	$F_{8, 25}$	$F_{4, 25}$
As	14.19***	120.15***	5.82***	0.68	1.18	1.59	0.51
Cd	0.04	30.18***	2.80**	6.81*	0.85	4.03***	1.53
Mn	10.74**	1.59	14.17***	0.22	1.75*	1.40	1.01
Cu	23.15***	24.57***	2.46*	5.25*	0.63	1.62	1.31
Zn	126.05***	22.29***	0.96	9.42**	0.94	1.47	1.37
Se	11.07***	64.78***	3.22**	8.94**	0.49	2.53*	1.34

Year	**WM**	**CH_4 (g m^{-2})**	**N_2O (g m^{-2})**	**CO_2 (g m^{-2})**	**GWP (g CO2eq m^{-2})**
2016	PFL	1.85 ± 0.66	0.007 ± 0.006	101.70 ± 12.90	53.66 ± 16.86
	AWD	0.17 ± 0.01	0.003 ± 0.003	42.91 ± 12.90	5.50 ± 0.56
2017	PFL	6.98 ± 1.05	-0.001 ± 0.000	161.08 ± 26.76	195.11 ± 29.53
	AWD	0.32 ± 0.16	-0.003 ± 0.003	90.73 ± 28.75	8.02 ± 4.94
ANOVA					
Factor		**$F_{1,8}$**	**$F_{1,8}$**	**$F_{1,8}$**	**$F_{1,8}$**
Year		17.68**	3.40	6.13*	17.55**
WM		44.34***	0.73	8.89*	46.86***
Y x WM		15.76**	0.030	0.07	16.35**

Name	**Acronym**	**Country of cultivation**	**Grain type**	**Cycle duration**	**Plant height**	**Yield**[¥]
ARELATE	ARE	France	Long A	Medium	Short	High
GAGERON	GAG	France	Round	Late	Short	Medium
GINES	GIN	France	Long B	Medium	Short	Medium
GLEVA	GLE	Spain	Round	Late	Short	High
JSENDRA	JSE	Spain	Round	Late	Short	Medium
LOTO	LOT	Italy	Long A	Early	Short	Medium
PUNTAL	PUN	Spain	Long A	Early	Short	Low
SELENIO	SEL	Italy	Round	Medium	Short	High
VIALONE NANO	VIA	Italy	Medium	Late	Medium	Low

[¥] Yield categories are based on Monaco et al. (2021) in which grain yield was assessed in a two-year field experiment. High, medium, and low-yielding cultivars refers to the grain yield in relation to the rest of the cultivars over the two years of the study: ARE and GLE consistently showed higher production than the remainder whereas the opposite is true for VIA.

		AWD						PFL					
		As	**Cd**	**Mn**	**Cu**	**Zn**	**Se**	**As**	**Cd**	**Mn**	**Cu**	**Zn**	**Se**
2016	**ARE**	0.15±0.034	0.008±0.002	20.154±4.099	4.922±0.553	61.115±7.778	0.067±0.014	0.237±0.03	0.005±0.002	17.773±3.55	3.068±0.479	51.765±6.736	0.055±0.012
	GAG	0.234±0.03	0.005±0.002	18.673±3.55	3.046±0.479	58.1±6.736	0.068±0.012	0.376±0.034	0.006±0.002	21.346±4.099	2.845±0.553	37.731±7.778	0.048±0.014
	GIN	0.186±0.06	0.004±0.004	14.065±7.099	2.745±0.958	55.659±13.471	0.086±0.024	0.271±0.042	0.007±0.003	18.555±5.02	3.022±0.678	43.561±9.526	0.065±0.017
	GLE	0.162±0.034	0.009±0.002	32.837±4.099	3.827±0.553	47.163±7.778	0.065±0.014	0.292±0.03	0.004±0.002	25.517±3.55	2.858±0.479	46.804±6.736	0.046±0.012
	JSE	0.142±0.042	0.008±0.003	31.967±5.02	3.302±0.678	72.155±9.526	0.047±0.017	0.293±0.034	0.004±0.002	29.354±4.099	2.589±0.553	41.366±7.778	0.026±0.014
	LOT	0.121±0.034	0.018±0.002	18.544±4.099	4.551±0.553	96.579±7.778	0.144±0.014	0.29±0.034	0.004±0.002	17.129±4.099	2.865±0.553	33.381±7.778	0.038±0.014
	PUN	0.185±0.034	0.005±0.002	16.646±4.099	2.503±0.553	67.625±7.778	0.048±0.014	0.259±0.03	0.01±0.002	18.063±3.55	3.562±0.479	46.155±6.736	0.054±0.012
	SEL	0.176±0.03	0.005±0.002	23.631±3.55	3.507±0.479	45.813±6.736	0.063±0.012	0.251±0.034	0.008±0.002	25.754±4.099	3.909±0.553	37.48±7.778	0.078±0.014
	VIA	0.201±0.03	0.013±0.002	32.481±3.55	3.173±0.479	59.478±6.736	0.067±0.012	0.373±0.034	0.007±0.002	25.405±4.099	2.267±0.553	36.838±7.778	0.026±0.014
2017	**ARE**	0.201±0.042	0.015±0.003	17.239±5.02	5.509±0.678	24.183±9.526	0.113±0.017	0.316±0.03	0.004±0.002	17.083±3.55	4.255±0.479	19.623±6.736	0.047±0.012
	GAG	0.315±0.03	0.013±0.002	29.841±3.55	4.801±0.479	22.375±6.736	0.115±0.012	0.433±0.042	0.004±0.003	14.376±5.02	2.075±0.678	15.609±9.526	0.049±0.017
	GIN	0.178±0.042	0.009±0.003	17.687±5.02	4.3±0.678	23.904±9.526	0.11±0.017	0.254±0.042	0.004±0.003	13.948±5.02	2.716±0.678	20.298±9.526	0.058±0.017
	GLE	0.192±0.06	0.01±0.004	40.078±7.099	4.35±0.958	25.641±13.471	0.104±0.024	0.311±0.03	0.004±0.002	40.703±3.55	4.479±0.479	23.468±6.736	0.055±0.012
	JSE	0.194±0.03	0.004±0.002	40.433±3.55	3.915±0.479	19.998±6.736	0.081±0.012	0.279±0.03	0.004±0.002	41.507±3.55	3.35±0.479	20.522±6.736	0.046±0.012
	LOT	0.188±0.034	0.015±0.002	17.682±4.099	5.826±0.553	24.373±7.778	0.121±0.014	0.429±0.03	0.004±0.002	20.186±3.55	3.588±0.479	19.167±6.736	0.058±0.012
	PUN	0.156±0.03	0.008±0.002	25.576±3.55	5.274±0.479	24.619±6.736	0.09±0.012	0.303±0.03	0.004±0.002	24.301±3.55	3.843±0.479	21.195±6.736	0.043±0.012
	SEL	0.235±0.034	0.006±0.002	20.531±4.099	4.467±0.553	22.057±7.778	0.095±0.014	0.37±0.03	0.004±0.002	28.935±3.55	3.801±0.479	18.627±6.736	0.058±0.012
	VIA	0.213±0.03	0.013±0.002	47.703±3.55	5.367±0.479	30.448±6.736	0.096±0.012	0.438±0.03	0.006±0.002	33.691±3.55	3.217±0.479	19.095±6.736	0.038±0.012

Declaration of interests

☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☒ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Maite Martinez-Eixarch reports financial support was provided by FACCE-JPI NET through Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria (INIA). Adam H. Price reports financial support was provided by FACCE-JPI NET through Biotechnology and Biological Sciences Research Council (BBSRC).

Multiple environmental benefits of alternate wetting and drying irrigation system with limited yield impact on European rice cultivation: the Ebre Delta case

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1. INTRODUCTION

Rice is a crucial crop for world food security as it is the staple food of more than half of the world's populations (Fairhurst and Dobermann, 2002). Apart from food provisioning, it provides a wide range of ecosystem services such as maintaining flora and fauna biodiversity, climate regulation, nutrient cycling, water purification, and cultural diversity and aesthetics (Settele et al., 2018; Nayak et al., 2019). However, paddy rice cultivation also leads to trade-offs or ecosystem disservices: it has large water (Bhatt, 2020) and carbon footprints (Zhang et al., 2018), and contributes to human uptake of heavy metals (Bouman et al., 2007). As one of the main sources of agricultural CH_4 emissions, paddy rice contributes to ca. 9 % to total anthropogenic greenhouse gas emissions (Saunois et al., 2016), while receiving 34 – 43 % of total world water irrigation (Bouman et al., 2007). Flooded soil conditions also favour accumulation of metalloids and heavy metals in the rice grain, such as arsenic (As) thus representing a potential health risk (Zhao et al., 2010). Therefore, it is imperative to implement and adopt cropping systems that enhance the beneficial effects of rice cultivation while minimizing the negative impacts.

The alternate wetting and drying system (AWD) is an irrigation technology for rice cultivation consisting in implementing alternate draining and flooded periods over the growing season either during the whole growth cycle or during certain growing stages. Multiple environmental benefits such as reduction in water consumption (Ye et al., 2013; Lampayan et al., 2015; Sriphirom et al., 2019; Wang et al., 2020), CH_4 emissions (LaHue et al., 2016; Peyron et al., 2016; Islam et al., 2020a; Wang et al., 2020) and As grain content (LaHue et al., 2016; Islam et al., 2018) are derived from AWD implementation but they are often coupled with yield losses (Carrijo et al., 2017). The extent of these environmental benefits is controversial as shown by their varying quantitative effect or by the trade-offs set with other covariables that can reduce or even negate the benefits. For example, the reported capacities to either mitigate CH_4 emissions or save water vary from *ca.* 30 % to more than 90 % (Linquist et al., 2015; Liang et al., 2016) and from 25 to 70 % (Ishfaq et al., 2020), respectively; the reduction in As grain content can be compensated by increases in cadmium (Cd) (Norton et al., 2017b), a health-hazardous heavy metal; whilst reductions in CH_4 can be partially or completely compensated by enhanced N_2O emissions resulting in an net increase of the global warming potential (GWP) (Lagomarsino et al., 2016; Kritee et al., 2018). The agronomic impact of AWD is also under debate with reported reduced (Linquist et al., 2015; Liang et al., 2016; Islam, 2018), unaffected yields (Carrijo et al., 2018; Runkle et al., 2019; Liao et al., 2020) or even enhanced rice production (Mofijul Islam et al., 2016) induced by soil aeration favouring root development (Zhang et al., 2009; Norton et al., 2017a)

The reasons of such a variability in the benefits/detriments of AWD are multiple but globally based on; firstly, the broad agronomic and environmental variability of rice agroecosystems with differing edaphic, climatic and agronomic conditions, and; secondly, the varying types of AWD regimes in terms of severity, *i.e.,* the critical threshold to which the water table is allowed to drop before reflooding (Linquist et al., 2015; Liang et al., 2016), and the moment of implementation (Boonjung and Fukai, 1996).

The varying outputs of AWD pose a limitation for its implementation so that a wide adoption of AWD in a rice growing area, wherein the pros are enhanced while the cons minimised, needs to be preceded by crop-context specific studies. AWD has been widely studied in Asian rice systems (Li and Barker, 2004; Lampayan et al., 2015; Mofijul Islam~~,~~ et al., 2016; Ishfaq et al., 2020) and in the USA (Linquist et al., 2015; LaHue et al., 2016; Carrijo et al., 2018). However, less is known about the potential benefits and trade-offs of AWD in Europe with the exception of Italy, where contrasting results on its GWP mitigation potential and yield impacts have been reported (Lagomarsino et al., 2016; Meijide et al., 2016; Monaco et al., 2021; Peyron et al., 2016; Oliver et al., 2019). To our knowledge, no studies have been conducted in Spain, despite being the second largest rice producing country in Europe.

Rice production in Spain accounts for 28% and 5% of the European production and crop extension, respectively. Rice in Spain is cultivated as a monocrop system with three periods with differing water managements: the growing (late April to September), post-harvest (October to December) and pre-growing (January to March) seasons. The standard water management during the growing season consists of fields permanently flooded to a depth of ca. 5 to 15 cm deep. In the post-harvest, the fields are left fallow and either maintained flooded or left to progressively drain, according to the either farmers' preferences or the agri-environmental schemes stablished in each growing region. Water supply is cut off in December hence the fields during the pre-growing season are dry in order to allow soil labouring. The irrigation water is derived from the river and supplied to rice fields through a huge canal network spread over the whole rice growing area.

A two-year field experiment was conducted in a representative Spanish rice growing area, the Ebre Delta. We tested the hypothesis that the benefits associated to AWD system, *i.e.,* mitigation of GHG emissions and reduction of grain element content, can be achieved within the context of a European rice crop system without compromising grain yield. In addition, the agronomic response to AWD of a set of representative European rice cultivars was assessed. This experiment was conducted in coordination to a parallel field study in Northern Italy (Monaco et al., 2021) with the overall goal of assessing the agronomic and environmental consequences of shifting the rice irrigation system from permanently flooded to AWD in rice cultivation in Europe.

2. MATERIAL AND METHODS

2.1 Site description

The study was conducted in 2016 and 2017 in the experimental rice fields of the Ebre Experimental Station of IRTA, located in the municipality of Amposta (40° 41’ 42’’N, 0 ° 47’ 00’’E) in the Ebre delta (Southern Catalonia, NE Spain). The climate of the region is Mediterranean with a mean annual precipitation of 500 mm, mostly distributed during spring and autumn. The mean annual air temperature is 18 ºC with mild winter (mean temperature in January 9 ºC) and summer (mean temperatures in July 24 ºC).

The soil texture (0 – 20 cm) of the field was silty clay loam (32.52 % clay; 64.5 % silt; 3 % sand) and the bulk density 1.3 g cm^{-3}. The pH was 8.3, and it contained 2.17 g kg^{-1} organic matter, 756 mg kg^{-1} of sulphates, 1.6 g kg^{-1} total nitrogen and 14.3 mg kg^{-1} Olsen phosphorous.

2.2 Crop management and experimental set-up

The experiment was laid out in a split-plot design, with four replicates. The main plots, of 250 m^2 each, represented the water management, including alternate wetting and drying (AWD) and permanent flooding (PFL), while the subplots, of 13.5 m^2 each, represented nine representative European rice cultivars from Spain: Gleva, Puntal and JSendra; Italy: Vialone Nano, Selenio and Loto; France: Arelate, Gines and, Gageron. The accessions were selected according to their representativeness and yield capacity, being all considered as high-yielding cultivars in their respective country. More information is provided in Supplementary table 1.

The AWD treatment was applied from the start of tillering until the start of flowering of the earliest variety to avoid spikelet sterility in any of the studied cultivars due to water-deficit stress imposed during the flowering (Lampayan et al 2015). This criterion was also followed by Norton et al. (2017a) and Carrijo et al., (2018). During the drying events, the water layer was left to progressively drop until a depth of approximately -20 cm (AWD threshold) and reflooded thereafter. Hydrological status of AWD plots was assessed by measuring both soil water potential with tensiometers (installed in all AWD plots at 25 cm depth) and water layer depth in piezometers (groundwater perforated PVC tubes of 150 cm long and 15 cm diameter, installed to a depth of 100 cm from the soil surface in all AWD plots).

Before and after AWD implementation, water management was the same in the two water treatments being the fields flooded at 5 to 10 cm deep and drained one month prior to harvest. In PFL, fields were permanently flooded at 5 to 10 cm deep throughout the growing season. The crop management was conducted following the standard practices of the area (Table 1). Soil labour operations were conducted in March in dry soil conditions, fields were flooded during the last week of April, and water seeded manually the first week of May. Each cultivar was independently harvested at the physiological maturity and when the grain moisture was around 20 – 21 %. The harvest was done over September and after that, the fields re-inundated before straw incorporation, in October, and left flooded until December.

2.3 GHG sampling and calculation of greenhouse emissions and global warming potential.

Closed opaque gas chambers (Altor and Mitsch, 2008) were used for gas sampling (more detailed description of the chambers and gas sampling procedure in Martínez-Eixarch et al., 2018). CH_4, CO_2 and N_2O were analysed simultaneously by a CG 7820A Agilent (USA) system equipped with a single channel and 2 valves of ten-port gas sampling with back-flush to vent and 6-port to change between the FID and micro-ECD detectors, using 2 packed columns Hayesep-Q 80-100 mesh 2 m x 1/8” × 2.0 mm Ultimetal Agilent (USA). Emission rates were calculated as the variation of gas concentration over the gas sampling in each chamber using linear interpolation. The increase of temperature in the headspace of the chamber was considered according to the ideal gas law. Only significant linear regressions ($p < 0.05$ and $R^2 > 0.80$) were accepted, and non-significant regressions were considered as zero emission rates. Cumulative GHG emissions between two consecutive sampling events were calculated assuming constant emission rates between them and then, they were all summed to calculate the seasonal cumulative CO_2, N_2O and CH_4 emissions. The overall global warming (GWP) effect , expressed in CO_2-equivalent units, was calculated considering a relative warming effect for CH_4 and N_2O 34 and 298 times higher, respectively, relative to CO_2 (Myhre et al., 2013).

Gas fluxes were only measured in Gleva cultivar subplots, in three out of the four replicates in each water treatment, and consistently within the same time window of 10.00 am to 1 p.m. During AWD implementation, gas sampling was conducted three times in each AWD cycle, that is at ca. -10 cm, -20 cm and +5cm water table depth, totalling 10 and 13 gas extractions over the 38 and 39-day period of AWD implementation in 2016 and 2017, respectively. Before and after AWD implementation, gas samples were collected on a bi-weekly and weekly basis, respectively. Simultaneously to gas sampling, soil parameters such as redox, electrical conductivity, temperature, and pH were measured next to the chambers at 10 cm soil depth.

2.4 Yield and yield-related traits

Grain yield and yield components per unit area were determined. Plant and panicle density per m^2 in each subplot were determined by counting panicle number in a 1-m^2 subarea (composite of four 0.25 m^2 subareas randomly placed in each subplot) at 4th leaf stage and heading. The remaining yield components, namely panicle size (spikelet number per panicle), spikelet fertility (filled grain number per panicle) and one-thousand grain weight, were calculated from 120 panicles randomly sampled in each plot. Separate grains in each plot were weighted and put in individual bags. Unfilled grains were separated by using a blower (Oregon Seed Blower) and then, spikelet fertility (number of filled grains per panicle) was calculated by dividing weight of filled grains by weight of total (filled and empty grains). Thousand-grain weight was calculated from the mean weight of six 500-grain samples whilst grain number per panicle from the average of panicle grain weight and the thousand-grain weight. Grain yield was determined from yield components. Plant height was measured in 4 randomly selected plants at the late milk phenological stage (77 BBCH). Indirect measurements of leaf nitrogen content were conducted using a chlorophyll meter, SPAD meter (Minolta Co.), with readings on 10 randomly assigned topmost fully expanded leaves (Cabangon et al., 2011).

2.5 Shoot and grain analyses

Fifteen productive tillers (stem + panicle) per subplot were randomly sampled at physiological maturity for determining grain and stem C and N content and grain heavy metal content, respectively. For the C and N content, dehusked rice grains and stems were oven dried (80 ºC), stems cut into 2-3 cm pieces and then a minimum of 0.3g of sample was randomly selected for ball milling. Milled powder of 0.006g was weighed into tin capsules. These samples were analysed on an Elemental Analyser (NA2500, Carlo Erba (CE) Instruments). Certified reference material (Beech Leaves [IRMM BCR®-100]) was used and repeated throughout the analysis as a quality control. For metalloids (As) and heavy metal content (Cd, Mn, Zn, Se and Cu), rice grains were dehusked and oven dried (80 ºC). For digestion, 0.2 g of dehusked grains were weighed out into 50 mL polyethylene centrifuge tubes. Grain samples were microwave digested (CEM Mars 5 microwave digester) with concentrated nitric acid and hydrogen peroxide as described in (Norton et al., 2012). Total element analysis (manganese, copper, zinc, arsenic, selenium, and cadmium) was performed by ICP-MS. Trace element grade reagents were used for all digests, and for quality control replicates of certified reference material (CRM) (Oriental basma tobacco leaves [INCT-OBTL-5], and rice flour [NIST 1568b]) were used; blanks were also included. All samples and standards contained 10 g L^{-1} indium as the internal standard.

2.6 Statistical analyses

A principal component analyses (PCA) was conducted to explore variability associated to year, water management and cultivar effect on yield, yield components and yield-related traits. To compare the agronomic, and As and heavy metal grain content response of the cultivars to water treatments, multivariate analyses of variance (MANOVA) was performed. MANOVA is used when several dependent variables are measured on each sampling unit instead of only one variable (for more details, see Rovira et al. (2012). Significances were further explored with three-way analysis of variance (ANOVA). Regarding the As and heavy metal grain content, when the concentrations were below the limit of detection (LOD), it was then assumed to be half of the LOD (Gu et al., 2020). The effect of water treatment of cumulative GHG emissions in Gleva cultivar was tested with ANOVA. Interannual variability was observed so that year was considered as a fixed factor in both MANOVA and ANOVA analyses. Statistical analyses were run with SPSS statistics software (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp).

3. RESULTS

The two-year mean temperature in winter (from December to February) and summer (from June to August) was 10.1 ± 0.5 ºC and 23.4 ± 0.4 ºC summer, respectively. Climatic conditions were similar over the two growing seasons, with mean temperatures and cumulative rainfall of 21.7 ± 1.4 ºC and 22.1 ± 1.2 and, 59.5 mm and 51.6 mm in 2016 and 2017, respectively.

3.1 Crop phenology and implementation of AWD and effects on soil redox

Tillering in Gleva started 45 and 36 days after sowing (DAS) in 2016 and 2017, respectively. The delay observed in the first year was probably caused by a post-herbicide shock which also provoked lower plant establishment, as later presented. Panicle initiation occurred 63 and 64 DAS, and heading, 88 and 83 DAS, in 2016 and 2017, respectively.

AWD was implemented from the start of tillering until flowering, *i.e.,* from 16th June to 29th July in 2016 and from 9th June to 27th July in 2017 (Fig. 1). Despite the targeted AWD threshold of – 20 cm, varying in-field AWD thresholds were finally registered (Table 2) mainly in terms of the soil water status readings.

Soil redox was increased by AWD but, regardless the water treatment, the ranges remained higher in 2016 (PFL: from - 194.9 ± 4.8 to + 66.5 ± 28.7 mV; AWD: from – 150.4 ± 13.6 to + 147.2 ± 0.0) than in 2017 (PFL: from – 258.4 ± 21.6 mV to – 146.3 ± 10.8; AWD: from – 188.0 ± 23.2 to – 12.0 ± 5.6). The critical range of soil redox values for methanogenic activity (– 100 mV to – 210 mV) was only achieved and established for a prolonged period in PFL fields in 2017.

3.2 Effect of AWD on canopy development, grain yield and yield-related traits

The crop establishment and crop growth in 2016 was significantly ($p < 0.05$) lower than in 2017 as indicated by the lower plant establishment (210.3 ± 8.2 *vs.* 252.9 ± 7.6 plants m^{-2}), plant height (84 ± 2 cm *vs.* 92 ± 2cm), canopy coverage (66 ± 9 % *vs*. 98 ± 2 %) and leaf N content at flowering (SPAD values: 38.5 ± 4.3 *vs.* 41.5 ± 35). The effect of water management on plant height and N leaf content across the cultivars and canopy development in Gleva (Fig. 2) was only significant in 2016: plant height ranged from 53 to 111 cm in AWD and from 63 to 122 in PFL, respectively, and the maximum canopy and SPAD values at flowering in PFL and AWD was 86 ± 6 *vs.* 47 ± 12 %, and 38.5 ± 4.3 *vs.* 37 ± 4 , respectively.

The PCA analysis conducted on yield and yield-related traits showed that most of the analysed variables were interdependent and significantly intercorrelated. The KMO measure of sampling adequacy (0.560) indicated the usefulness of the PCA, with the first two components explaining 41.2 % of the total variation (Fig. 3). The first PCA axis showed the associations between N content and yield-related traits. Nitrogen in shoots and grains at maturity were negatively correlated to N content in leaves at flowering (SPAD), C:N ratios in grain and shoots at maturity and, sink (spikelet number per m^2) and source (filled grain number per m^2) strength. The second PCA axis showed a negative correlation between yield and most of the yield components, and plant height and plant density. The distribution of the point scores in the PCA biplot presented two groups separated by the year: rice plants in 2016 showed lower leaf N content at flowering but large N content in shoot and grains at maturity than in 2017. The same is true for plants submitted to AWD in 2016 whereas no differentiated distribution along the PCA1 attributable to water management was observed in 2017. No specific distribution by cultivars along the two PCA axes was observed.

The multivariate analyses of variance (MANOVA) test showed an overall significant effect of water management (Wilks's $\lambda = 0.447$; $F_{7,21} = 17.85$; $p < 0.0001$), cultivar (Wilks's $\lambda = 0.001$; $F_{56,549} = 28.66$; $p < 0.0001$), year (Wilks's $\lambda = 0.304$; $F_{7,101} = 33.03$; $p < 0.0001$), and year × cultivar (Wilks's $\lambda = 0.227$; $F_{56,549} = 3.10$; $p < 0.0001$) , water management × year (Wilks's $\lambda = 0.551$; $F_{7,101} = 11.74$; $p < 0.0001$), water management × cultivar (Wilks's $\lambda = 0.316$; $F_{56,549} = 2.34$; $p < 0.0001$) and the water × cultivar x year interactions (Wilks's $\lambda = 0.415$; $F_{56,549} = 1.74$; $p < 0.001$) on yield and yield components.

Specifically, for yield and each yield-related trait, the results of MANOVA test as well as the mean marginal means are presented in Table 3. The overall mean annual grain yield in both years of the study was similar (chronologically: 7358 ± 207 *vs.* 7423 ± 211 kg ha^{-1}). Under PFL, grain yield in 2016 was slightly higher, but non-significantly ($p = 0.31$) than in 2017 (8106 ± 294.8 *vs.* 7577 ± 317 kg ha^{-1}) while the opposite was true in AWD(chronologically: 6590.0 ± 229.7 vs 7268.4 ± 282.1 kg ha^{-1}).

Averaged across the years and cultivars, AWD reduced grain yield 12 %; by years, the impact of AWD was larger and significant in 2016 (19 %) than in 2017 (6 %), being statistically insignificant.

The three-way effect of the studied factors, including year as fixed factor, revealed contrasting cultivar responsiveness to AWD in terms of both severity (magnitude) and direction over the two years of the study. The yield response to AWD across the cultivars is presented in Fig. 4. LOT was tolerant to AWD as shown by the similar grain yields in both water treatments over the two-year study. GAG, GIN, GLE, JSE consistently performed as sensitive cultivars to AWD with yield declines of 24 % (range: 20 % – 27 %) and 12 % (range: 10 % – 13 %) on average in 2016 and 2017, respectively, though the latter being non-significant. SEL showed similar yield loss under AWD than the sensitive group in 2016, *ca.* 24 %, but maintained the same yield in 2017. Finally, ARE, VIA, PUN showed contrasting interannual responses to AWD. VIA increased grain yield by 51 % under AWD in 2016 but then it declined by 8 % in 2017; ARE was affected by AWD by 23 % in 2016 but then, in 2017, it turned to perform 14 % better than PFL; PUN sharply declined grain yield by 37 % under AWD in 2016 but remained stable in 2017.

The response to AWD of yield components and yield-related traits were investigated to explore how AWD modulated yield formation (Table 3). Panicle density in LOT remained unaffected by AWD whereas it declined in GLE, GAG, GIN and JSE, by 6 – 7 %, and by 6 % and 21 % in SEL in 2016 and 2017, respectively. The number of spikelets per panicle (panicle size) was largely affected by AWD in 2016, with 21 % to 27 % in GLE, GAG, GIN, JSE, SEL and LOT, but to a lesser extent, 3 % to 4 %, in 2017. Sink size, which is the number of spikelets per m2, was reduced by 25 % in GAG, GIN, GLE, JSE and SEL in 2016 and by 12 % in LOT while in 2017 the reduction ranged from 4% to 6%. Rates of grain filling increased in 8 and 4 percentage points in LOT in 2016 and 2017, respectively, but in less than 3 percentage points in GLE, GAG, GIN, JSE and SEL in 2016 and 2017.

VIA, PUN, and ARE showed contrasting responses to AWD in the two years of the study. In 2016, PUN and ARE reduced panicle density (46 % and 15 %, respectively) and panicle size (33 % and 21 %, respectively) resulting in reduced sink size (48 % and 30 %, respectively). Thereafter, spikelet fertility was increased by ten percentage points in both of them. By contrast, none of the yield components was affected by AWD in 2017 in both cultivars. In 2016, VIA increased panicle density, panicle fertility and thousand-grain weight under AWD but reduced panicle size whereas in 2017, panicle size, panicle fertility and a thousand-grain weight were reduced while panicle density increased.

Regarding the overall cultivar performance, JSE consistently ranked among the best grain yielding cultivars within each year and water management, even with the yield declines suffered. The cultivars JSE, PUN, GLE and SEL, GAG showed the best performance consistently across the years and within each water management. By contrast, VIA showed the worst performance.

3.3 Effect of water management and cultivar on Arsenic and heavy metal content

The multivariate analyses of variance (MANOVA) test showed an overall significant effect of water management (Wilks's $\lambda = 32.00$; $F_{6,74} = 40.10$; $p < 0.0001$), cultivar (Wilks's $\lambda = 4.83$; $F_{48, 368} = 4.83$; $p < 0.0001$), year (Wilks's $\lambda = 0.316$; $F_{6,74} = 26.70$; $p < 0.0001$), year x water management (Wilks's $\lambda = 0.70$; $F_{6,74} = 5.28$; $p < 0.0001$) and water management x cultivar (Wilks's $\lambda = 0.42$; $F_{48,368} = 1.46$; $p < 0.05$) on the element concentration in the grains of rice. Marginal means (± SE) can be found in Supplementary table 2.

The grain concentration of As (Fig. 5, Table 4) ranged from 0.121 to 0.438 mg As kg^{-1}, being significantly ($p < 0.001$) larger in 2017 (0.28 ± 0.01 mg As kg^{-1}) than in 2016 (0.23 ± 0.01 mg As kg^{-1}). Globally, AWD decreased As concentration in all genotypes (Table 4, Fig. 5) by ca. 40 % in the two years (PFL vs AWD: 2016; 0.29 ± 0.01 *vs.* 0.17 ± 0.001; 2017, 0.35 ± 0.01 *vs.* 0.21 ± 0.01 mg As kg^{-1}.

Under PFL, all the cultivars surpassed the threshold of 0.20 mg As kg^{-1}, while the cultivars GAG and VIA (and LOT in 2017) consistently showed larger grain As than the remainder. Under AWD, the cultivars GIN, GLE, JSE, LOT and PUN maintained the grain As below 0.2 mg kg^{-1} consistently in both years of the study and GAG still showed the largest As concentration in both years.

The overall grain Cd concentration (Table 4, Fig. 5) was similar in the two years of the study (0.007 ± 0.001 mg Cd kg^{-1}) as well as the increase in AWD (PFL *vs.* AWD: 0.005 ± 0.001 *vs.* 0.009 ± 0.001 mg Cd kg^{-1}), though the concentration remained low in both treatments (< 0.018 mg Cd kg^{-1}). In 2016, Cd concentration in the cultivars ARE, GAG, PUN, SEL and VIA under AWD felt below the limit of detection (and so half of the sample LOD was used for statistical analyses, see Material and Methods). No specific cultivar pattern was observed for Cd concentration, safe LOT and VIA showing the largest concentrations under AWD (0.013 – 0.018 mg Cd kg^{-1}).

The concentration of Mn was not affected by the water management (24.93 ± 0.87 mg Mn kg^{-1}) whereas the genotype and genotype -by year effects were significant, being VIA, GLE and JSE the cultivars consistently showing the largest grain Mn concentration 29 – 41 mg Mn kg^{-1}) while LOT, GIN and ARE the lowest (<22 mg Mn kg^{-1}).

Cu, Se and Zn concentrations increased with AWD by 27 %, 78 % and 41 % respectively, with the water -by year effect significant which was explained by the consistent increase in both years but only significant in one of them. The cultivar effect was significant for Cu and Se.

3.4 Effects of AWD on GHG emission rates and cumulative GHG

CH_4 emissions in Gleva cultivar (Fig. 6) under the standard water management, *i.e.*, permanent flooding, were very low in 2016, showing a mean seasonal rate of 0.22 ± 0.12 mg CH_4 m^{-2} h^{-1} and totalling 1.85 ± 0.7 g CH_4 m^{-2} emitted over the growing season. In 2017, the mean seasonal rate of

CH_4 emissions under PFL was 2.45 ± 0.3 mg CH_4 m^{-2} h^{-1} totalling 7.0 ± 1.1 g CH_4 m^{-2} which was aligned with the emissions previously reported in the area by Martínez-Eixarch et al (2018, 2021). Similarly, more CO_2 was emitted in the second year of the study under the standard water management (Table 5) whereas negligible N_2O emissions were found in the two years (<0.01 g N2O m^{-2}).

AWD significantly reduced mean emission rates of CH_4 by 79 % and 94 % in comparison to PFL, in 2016 and 2017, respectively, leading to reduction in cumulative CH_4 emissions of 91 % to 95 % (Table 5). N_2O emissions rates (Supplementary Figure 1) were very low in the two water managements over the growing season though contrasting effects of AWD were found over the two years of the study: in 2016, mean emission rates were slightly reduced by 14 % in 2016, leading to 58 % less cumulative N_2O, but increased by 600 % in 2017, leading to 300 % more cumulative emissions (Table 5). N_2O fluxes were detected ten days after the fertilization events in AWD in 2016 ,10.5 ± 10.5 µg N_2O m^{-2} h^{-1}, and in both AWD and PFL in 2017, 2.5 ± 2.5 and 1.9 ± 1.3 µg m^{-2} h^{-1}, respectively. AWD significantly reduced mean emission rates of CO_2 by 58 % and 13 % in comparison to PFL, in 2016 and 2017, respectively (Supplementary Figure 1), leading to reduction in cumulative CO_2 emissions of 58 % and 44 % (Table 5) .

The resulting GWP (CH_4 + N_2O) was significantly reduced by AWD by 90 % and 96 %, in 2016 and 2017, respectively (Table 5). Therefore, the mitigation potential of AWD by reducing CH_4 emissions was not offset by enhanced N_2O emissions. CH_4 was the main contributor of GWP (> 90 %) in all the treatments.

4. DISCUSSION

4.1 Agronomic performance of the rice crop: interannual variability under permanently flooded fields

Plants grown in both water managements in 2016 presented symptoms of phytotoxicity caused by the application of the herbicide, namely reduced plant establishment, N content in leaves (SPAD), crop canopy and plant height (Jason et al., 2007; Awan et al., 2016). Plants under PFL could recover from the injury, as indicated by the comparable grain yield to that obtained in 2017. By contrast, the herbicide-induced impact apparently persisted and yet was aggravated by the implementation of AWD as indicated by the poor canopy development (Fig. 2).

4.2 Agronomic response to AWD

The present study examines the response of nine representative European cultivars submitted to AWD system. Overall, AWD implemented in 2016 was more severe than in 2017, since 7 out of 9 cultivars significantly reduced grain yield, whereas in the second year, only non-significant declining trend was observed in the cultivars JSE, GLE, GIG and GAG and VIA. The stronger severity of AWD in 2016 was likely explained firstly, by the weaker health conditions of the plants prior AWD implementation caused by the herbicide phytotoxicity and, secondly, by the excessive drought (– 25 ± 0 cm or – 54 ± 14 KPa) imposed around panicle initiation rather than by a repetitive exposure to water stress along the AWD cycles. It is then derived from this that the timing of AWD thresholds is decisive for yield response to water stress. Hereafter, AWD implemented in 2016 and 2017 will be referred as severe and mild AWD, respectively.

The present study revealed contrasting genotype response to AWD which is aligned with Bueno et al. (2010), and Liang et al. (2016) but contrasts with Zhang et al. (2009), Yang and Zhang (2010) and, Norton et al. (2017b). LOT was the only cultivar identified as tolerant to AWD, in agreement with Orasen et al. (2019) but in contrast with Miniotti et al. (2016). The group formed by JSE, GLE, GAG and GIN and SEL was sensitive to severe AWD whilst it showed a non-significant declining trend under mild AWD. ARE and PUN performed as highly sensitive to severe AWD, but tolerant to mild

AWD. Finally, VIA showed opposite responses in the two years of the study. The erratic response of these three cultivars (VIA, ARE and PUN) prevents drawing conclusions on their sensitivity to AWD.

Differential sensitivity of the cultivars to AWD during the growing stages across the cultivars was detected. Panicle size was consistently affected by severe AWD in all cultivars indicating that early reproductive stages are sensitive to AWD thresholds around – 25 cm or – 50 KPa but tolerant to – 19 KPa and – 20 cm. Similarly, Liang et al. (2016) found differing responsiveness of panicle size to contrasting severities of AWD. While severe AWD consistently affected panicle size, varying genotype responsiveness of panicle density severity was found. Reductions in panicle density can be given by either reduced tillering ability (Boonjung and Fukai, 1996; Martínez-Eixarch et al., 2015) and/or enhanced tiller abortion (Okada et al., 2002; Alou et al., 2018). In ARE and PUN, panicle exertion induced by water stress around panicle initiation in 2016 could have reduced panicle density (Okada et al., 2002). On the other hand, capacity is apparently resistant to AWD-induced stress, as indicated by the non- response in 2017, when soil during the vegetative stage was even drier than in 2016. Instead, the comparable reduction of panicle density of GLE, JSE, GAG and GIN over the two years suggests that tillering capacity, rather than either tiller abortion or constrained panicle exertion, was the main driver of this reduction. Contrasting with Zhang et al. (2009) and Norton et al. (2017), none of our studied cultivars seemed to present enhanced tillering ability under AWD.

Therefore, yield loss was explained by the cumulative effect of AWD on both panicle density and panicle size, that is sink size, and by the subsequent incapacity of the plants to sufficiently compensate such an impact by increasing grain filling rates. Indeed, this compensatory mechanism conferred the consistent tolerance to LOT under the two severities of AWD. Bueno et al. (2010) also pointed out strong compensatory mechanisms as the drivers of AWD tolerance in some cultivars.

VIA, which is a tall cultivar, was benefitted from severe AWD by reducing plant height (plant height in PFL and severe AWD: 103 ± 3 vs 118 ± 2 cm, data not shown) (Wang et al 2016) thereby conferring lodging resistance and, eventually favouring grain yield (Setter et al., 1997; Wang et al., 2012).

To summarize this agronomic section, severe AWD with drying events lower than – 50 KPa around early reproductive stages, cause substantial yield reductions. Yield penalties under severe AWD (< -50 KPa) have been reported elsewhere (Bueno et al., 2010; Yang and Zhang, 2010) but others found no effect (Carrijo *et al.* 2018). The milder version of AWD implemented in the second year of the study, consisting in keeping a critical mean AWD threshold of –25 KPa over the AWD cycles and, specifically of – 20 KPa around panicle initiation, does not have a significant impact on production. Despite this, some varieties showed a downward trend that we believe can be overcome with a critical threshold of –20 KPa overall the AWD implementation (Bouman et al., 2007). To achieve this, it is very important to have a good control of the soil hydrology as sudden drops in water potential can occur in a few days and have serious consequences for production. In addition, development of rice varieties adapted to AWD, that is with as high yields as the best high yielding variety under PFL, would contribute to this pursuit and to widening the adoption of AWD water management by farmers (Price et al., 2013; Volante et al., 2017). In our study, the AWD-reduced yields in the Spanish cultivars were on average larger than the remainder, likely because of their better adaptation to the local growing conditions.

4.3 AWD effects on concentration of Arsenic and heavy metal content in grain

The averaged As concentration in rice grains under permanent flooding conditions was 0.32 mg As kg^{-2}, which surpasses the FAO recommendation of 0.2 mg kg^{-1} (Codex Alimentarius Commission, 2014). This concentration is comparable to the levels reported in Arkansas (Norton et al., 2012;

Linquist et al., 2015) but lower than those in Texas (Norton et al., 2012) and higher than in Philippines (Islam et al., 2020) and California (Lahue et al., 2016).

Soil redox influences on As speciation and mobilisation in paddy soils thus mediating plant As uptake and subsequently the grain As concentration. Soils with low redox potential favour As solubility and thereby plant uptake, as reviewed by Meharg and Zhao (2012). Therefore, the more reducing conditions in the permanently flooded plots in 2017 explains the larger As content in grains.

Our study provides further evidence that the implementation of AWD significantly reduces As content in grain. In the case of Ebre Delta, 40 % of grain As reduction was achieved which allowed keeping the mean threshold below the upper limit recommended by FAO and the European Commission. This AWD-induced reduction is in accordance with other studies, although with wide overall variation, from 18 % to 63 % (Linquist et al., 2015; LaHue et al., 2016; Norton et al., 2017), attributable to genotype or site-specific rice cultivar physiology (Wu et al., 2011; Norton et al., 2012; Rai et al., 2015), timing and severity of AWD implementation (Linquist et al., 2015; Norton et al., 2017a; Carrijo et al., 2018; Carrijo et al., 2019), application of organic matter (Islam et al., 2020). The multifactorial nature of grain As response to water management highlights the need of site-specific studies to evaluate the potential of AWD to reduce grain As. Further, the present study reveals genetic variation in As concentration in rice grains across the most representative European cultivars, which is in line with previous research (Norton et al., 2009; Ahmed et al., 2011; Norton et al., 2017). In our field study, GAG consistently presented the highest As concentration under both water treatments in the two years whilst 5 out of the 9 cultivars (GIN, GLE, JSE, LOT and PUN) kept As concentration below the recommended threshold under AWD. The genotype effect can be driven by both the differing root anatomy modulating root radial oxygen loss (ROL) and iron plaque formation, which is related to As speciation and thus, As bioavailability (Mei *et al.*, 2012), and by differing abilities on uptake and/or partitioning As to grains (Suriyagoda et al., 2018). In addition, these processes can also be modulated by environmental and agronomic factors so that a genotype-by- environmental interaction, found in other studies (Norton 2009; Norton et al., 2010; Pillai 2010; Ahmed 2011) could be expected.

In contraposition to As, grain Cd increased with AWD by 80 % 2017, but remained below the maximum levels of 0.2 mg kg^{-1} determined by the European Commission (Commission Regulation (EC) No 629/2008) in both AWD and PFL. The levels found in our study fall within the range previously reported in Ebre Delta (Roig et al., unpublished) and in Bangladesh (Norton et al., 2017a; Norton et al., 2017b). The mobility and availability of Cd in paddy fields is related to soil redox potential so that the reducing environment promotes the reduction of sulfates to sulfides to which Cd^{2+} ions are bound thereby reducing its bioavailability (Rinklebe *et al.*, 2016). The formation of sulfides in our fields under the lowest the soil redox potential is assumed and supported by the soil sulphate content of 756 mg kg^{-1}. The insignificant effect of water management in 2016 could be explained by the number of cultivars showing Cd concentrations below the limit of detection. Despite the consistent overall trend of increasing Cd content across the cultivars, such an effect was only significant in some cultivars: ARE in 2016 and 2017 and, ARE, LOT and VIA in 2017.

No effect of water on Mn was detected, which is in contrast with Norton et al. (2017a) who found increased grain Mn under AWD. The stability of the Mn in soils is regulated by both redox potential and pH (Reddy and DeLaune, 2008). In alkaline soils the solubility of Mn is low because it can either coprecipitate with carbonates forming solid $MnCO_3$ under reducing conditions or remain present as solid Mn (IV) in the form of MnO_2 under oxidizing conditions (Pan et al., 2014). Therefore, the basic soil of our field experiments (pH=8.3), in contraposition with the acid soils in Norton's study, could explain the contrasting responsiveness of grain Mn content to water management. The significant genotype effect was also found by Norton et al. (2017b).

The grain Cu concentration is comparable to a previous research in Ebre Delta (Roig et al., unpublished). The increasing effect of AWD on Cu and Se is in agreement with Norton et al. (2017a, 2017b) and Zhou et al. (2018), respectively, while that on Zn agrees with Wang et al. (2014) but not with Norton et al. (2017b) who found no AWD response. The AWD-induced increase in Cu, Se and Zn deserves further research as they all are important elements for plant health and human nutrition (White and Broadley, 2009; White and Brown, 2010)

4.4 Greenhouse gas emission and GWP

The plausible herbicide phytotoxicity shown in the first year of the experiment, influenced both rice physiology and the system of plant-soil-atmosphere gas exchange that led to reductions in CH_4 and CO_2 emissions in Gleva.

Reduced CO_2 emissions in 2016 likely resulted from reduced foliar coverage, thus reducing area for gas exchange, leaf N content (Reich et al., 2008) and presumably stomatal conductance caused by N shortage (Hirasawa et al., 2010). They also indicate a net reduction of the ecosystem respiration, including heterotrophic and autotrophic respiration. Oliver et al. (2019) found, in a parallel field experiment in Italy, that ecosystem respiration was dominated by autotrophic respiration in relation to heterotrophic respiration, so that changes in the foliar coverage could have been the main driver of reduced CO_2 emissions. Nevertheless, reduced heterotrophic respiration could have also resulted from inhibited plant growth and rhizoexudate release caused by N shortage thereby limiting carbon substrate for soil microorganisms.

The emissions of CH_4 in 2016 under the standard water managements were unexpectedly lower than those previously reported in rice fields in Ebro Delta (Martínez-Eixarch et al., 2018; Martínez-Eixarch et al., 2021). The low emissions are coherent with the interval of soil redox potential recorded throughout the growing season (–194.9 ± 4.8 to + 66.5 ± 28.7 mV) remaining mostly outside of the critical threshold for methanogenesis (Eh< – 150 mV; (Hou et al., 2000); Sahrawat 2005). Three processes mediated by the development of canopy and its positive association with the vegetative growth (Dingkuhn et al., 1999, 2000) could explain reduced CH_4 emissions. The first is a limited transport of CH_4 from the soil to the atmosphere via rice stems, since plant mediated transport is the major pathway of CH_4 diffusion (Aulakh et al., 2000; Pittelkow et al., 2013). The second, low crop development indicate low release of rhizoexudates and so reduced availability of labile organic matter, which is the predominant source for methanogenesis via DOC (Aulakh et al., 2001; Kögel-Knabner et al., 2010). The third, is that low N availability limits methanogenesis as supported by the positive association between rates of N fertilization rates and CH_4 emissions in Ebro delta rice fields (Martínez-Eixarch et al., 2021) and the conclusions extracted from meta-analyses by Liu and Greaver(2009) and Banger et al. (2012).

Our study provided further evidence that AWD can significantly reduce both CH_4 and GWP of paddy rice which is aligned with past studies (Linquist et al., 2015; Lahue et al., 2016; Runkle et al., 2019; Islam et al., 2020; Wang, 2020) ~~;)~~ but in contrast with others reporting increases of net GWP driven by increased N_2O emissions during the aeration events eventually offsetting CH_4 mitigation (Lagomarsino, 2016; Krittie, 2018; Liao et al., 2020;). In our study, even the increased N_2O found in 2017 could not compensate the decline in CH_4. No fluxes of N_2O during the aeration events were observed, only seven days after the fertilization in 2016 and with low values, suggesting that the nitrification process was likely completed to N_2 production, and that an appropriate N management was implemented, consisting in reflooding the fields the day after the topdressing N application since N_2O peaks in intermittent irrigation are linked to N fertilization events (Peyron et al., 2016; Lagomarsino et al., 2016). Further, the range of redox potential found in AWD fields mostly felt within the optimum window of soil redox potential (from +120 to –170 mV) in paddy soils around pH neutrality, in which both CH_4 and N_2O emissions are minimized (Yu et al., 2001; Yu and Patrick, 2003). This could also explain the large CH_4 mitigation capacity of AWD in our studied conditions, around 90 %, which is comparable to that reported by Linquist et al. (2015) and Peyron et al. (2016). Another plausible explanation for such a large CH_4 mitigation capacity would be that CH_4 fluxes did not completely recover after AWD cycles in relation to those in PFL, as also observed by Linquist et al. (2015), suggesting a lag phase for methanogenesis after the aeration periods. However, it should be noted that the low frequency of GHG sampling from the last AWD cycle to the maturity could have also underestimated CH_4 emissions during this period. More frequent sampling beyond the period of AWD implementation is necessary to precisely estimate its mitigation capacity.

5. CONCLUSIONS

This study confirms that AWD can be safely implemented with limited or null yield impact while obtaining the associated environmental benefits, namely, reduced CH_4 emissions and As content in grain. The safe version of AWD consists in implementing this water management from tillering to flowering and keeping the critical AWD threshold at –20 cm, with special attention around panicle initiation, which was the most sensitive growing stage.

The cultivar effect on AWD response was significant. The cultivar LOT was tolerant to AWD, even to a severe version of AWD, being such a tolerance conferred by the strong capacity to compensate reduced panicle size by enhancing grain filling rates. The slight non-significant yield decline found in the group formed by GLE, JSE, GAG and GIN under mild AWD can likely be overcome by a precise implementation of safe AWD.

AWD significantly reduces CH_4 emissions and the GWP by up to 90% being the large mitigation capacity explained by the negligible N_2O emissions found in both water treatments.

Our study provides further evidence that the implementation of AWD significantly reduces As content in grain, by 40 % in the case of Ebre Delta rice growing conditions, thus allowing to keep the mean threshold below the upper limit recommended by the European Commission. By contrast, Cd increases by 20 % with AWD, though the concentration remained below the maximum allowed levels.

The present is the first comprehensive study of the implementation of the AWD irrigation system in a Europe rice growing system in which climate change mitigation (GHG emissions), food safety (metalloid content in grain) and agronomic (yield) factors have been examined under an integrative approach. Moreover, the analyses of the genotype x management interaction, based on a set of the 9 most representative European cultivars, provides new knowledge transferrable either to breeding programs so that it can be exploited to better utilize European collection of germplasm, or to the rice farm sector to facilitate a safely implementation of this system avoiding risks of yield reduction. Altogether represents a step forward towards to the implementation of a more sustainable economically and environmentally rice cultivation in Europe.

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CAPTIONS

Table 1. Crop management in experimental fields in the two years of the study (2016 – 2017)

Table 2. Minimum water table depth and soil water status (AWD thresholds) achieved at each wetting and drying cycle over AWD implementation in 2016 and 2017. Data represent mean across the four replicates ± SE.

Table 3. Marginal means (± standard error) of yield, and yield-related traits (above) in both water treatments, AWD and PFL, in the nine European rice cultivars in the two years of the study (2016 – 2017) and (below) MANOVA of the effect of water management (WM), cultivar (CV) and year (Y) and their interactions on yield and yield-related traits. The asterisks indicate significance of the factor at 0.05 (*), 0.01(**) and 0.001 (***) levels. Abbreviations. Rice genotypes: ARE, Arelate; GLE, Gleva; PUN, Puntal; GAG, Gageron; JSE, JSendra; SEL, Selenio; GIN, Gines; LOT, Loto; VIA, Vialone Nano. Yield and yield-related traits: GYL, grain yield (kg ha^{-1}); PND, panicle density; TGN, total number of spikelets per panicle; TGW, one thousand-grain weight (g); FER, panicle fertility (%filled grains per panicle); SINK, sink strength (grain number per m^2).

Table 4. MANOVA of the effect of water management, cultivar and year and their interactions on the grain element concentration. Abbreviations: Y, year; WM, water management; CV, cultivar. The asterisks indicate significance of the factor at 0.05 (*), 0.01(**) and 0.001 (***) levels.

Table 5. Marginal means (± standard error) of the seasonal CH_4, N_2O, CO_2 and GWP in both water treatments, AWD and PFL, in Gleva cultivar in the two years of the study (2016 – 2017) and ANOVA of the effect of water management (WM), year (Y) and their interaction (WM x Y). The asterisks indicate significance of the factor at 0.05 (*), 0.01(**) and 0.001 (***) levels.

Table S1 Main agronomic traits of the studied cultivars.

Table S2 Marginal means (± standard error) As and heavy metal concentration in both water treatments, AWD and PFL, in the nine European rice cultivars in the two years of the study (2016 – 2017)

Figure 1. Water regime of experimental rice plots under AWD and PFL in the two years of the study (2016 and 2017). Arrows indicate the date of panicle initiation (chronologically: 63 and 64 days after DAS) and flowering (chronologically: 88 and 83 DAS) in Gleva cultivar. AWD was implemented from 16th June 2016 (45 DAS) to 29th July 2016 (88 DAS) and from 9th June 2017 (36 DAS) to 27th July 2017 (84 DAS).

Figure 2. Canopy development in Gleva cultivar over the rice-growing period in AWD and PFL treatments in the two years of the study (2016 – 2017).

Figure 3. Principal component analyses (PCA) of grain yield and yield related traits. A) Factor loadings of the variables and, B) samples scores showing the distribution of water management, AWD (solid circles) and PFL (empty circles), and the cultivars. Abbreviations. Rice cultivars: ARE, Arelate; GLE, Gleva; PUN, Puntal; GAG, Gageron; JSE, JSendra; SEL, Selenio; GIN, Gines; LOT, Loto; VIA, Vialone Nano. Agronomic traits: Grain_N, grain N content; Shoot_C, shoot C content; Shoot_N, shoot N content; Grain_C, grain C content; PN_PL, panicle number/plant; FER, panicle fertility; PND, panicle density; StH, time from sowing to heading; TGW, thousand-grain weight; HGT, plant height; PLD, plant density; GYL, grain yield; SINK strength (grain number m^{-2}); SOURCE strength (filled grain m^{-2}); GrainCN, grain C:N; ShootCN, shoot C:N ratio; SPAD at flowering.

Figure 4. Marginal means (± standard error) of yield in each cultivar under AWD and PFL in the two years of the study (2016 and 2017). Error bars indicate the standard error of the marginal mean. Abbreviations. Rice genotypes: ARE, Arelate; GLE, Gleva; PUN, Puntal; GAG, Gageron; JSE, JSendra;

SEL, Selenio; GIN, Gines; LOT, Loto; VIA, Vialone Nano. The rectangles indicate the cultivars with consistent interannual pattern across the years: the tolerant cultivar LOT and the sensitive cultivars JSE, GLE, GIN, GAG.

Figure 5. Marginal means (± standard error) of grain concentration of As (above) and Cd (below) under the studied water treatments, AWD and PFL, in nine representative European rice cultivars, in the two years of the study (2016 – 2017). Abbreviations. Rice cultivars: ARE, Arelate; GLE, Gleva; PUN, Puntal; GAG, Gageron; JSE, JSendra; SEL, Selenio; GIN, Gines; LOT, Loto; VIA, Vialone Nano. Note that the order of the cultivars in the X-axis and the scale in the Y-axis are different for As and Cd figures.

Figure 6. Mean emission rate of CH_4 (mg m^{-2} h^{-1}) over the growing in the two studied water treatments, AWD and PFL, over the two years of the study (2016 – 2017). The bars represent the standard error of the mean.

Figure S1 Mean emission rate of (above) N_2O (µg m^{-2} h^{-1}) and (below) CO_2 (mg m^{-2} h^{-1}) over the growing in the two studied water treatments, AWD and PFL, over the two years of the study (2016 – 2017). The bars represent the standard error of the mean.

Multiple environmental benefits of alternate wetting and drying irrigation system with limited yield impact on European rice cultivation: the Ebre Delta case

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1. INTRODUCTION

Rice is a crucial crop for world food security as it is the staple food of more than half of the world's populations (Fairhurst and Dobermann, 2002). Apart from food provisioning, it provides a wide range of ecosystem services such as maintaining flora and fauna biodiversity, climate regulation, nutrient cycling, water purification, and cultural diversity and aesthetics (Settele et al., 2018; Nayak et al., 2019). However, paddy rice cultivation also leads to trade-offs or ecosystem disservices: it has large water (Bhatt, 2020) and carbon footprints (Zhang et al., 2018), and contributes to human uptake of heavy metals (Bouman et al., 2007). As one of the main sources of agricultural CH_4 emissions, paddy rice contributes to ca. 9 % to total anthropogenic greenhouse gas emissions (Saunois et al., 2016), while receiving 34 – 43 % of total world water irrigation (Bouman et al., 2007). Flooded soil conditions also favour accumulation of metalloids and heavy metals in the rice grain, such as arsenic (As) thus representing a potential health risk (Zhao et al., 2010). Therefore, it is imperative to implement and adopt cropping systems that enhance the beneficial effects of rice cultivation while minimizing the negative impacts.

The alternate wetting and drying system (AWD) is an irrigation technology for rice cultivation consisting in implementing alternate draining and flooded periods over the growing season either during the whole growth cycle or during certain growing stages. Multiple environmental benefits such as reduction in water consumption (Ye et al., 2013; Lampayan et al., 2015; Sriphirom et al., 2019; Wang et al., 2020), CH_4 emissions (LaHue et al., 2016; Peyron et al., 2016; Islam et al., 2020a; Wang et al., 2020) and As grain content (LaHue et al., 2016; Islam et al., 2018) are derived from AWD implementation but they are often coupled with yield losses (Carrijo et al., 2017). The extent of these environmental benefits is controversial as shown by their varying quantitative effect or by the trade-offs set with other covariables that can reduce or even negate the benefits. For example, the reported capacities to either mitigate CH_4 emissions or save water vary from *ca.* 30 % to more than 90 % (Linquist et al., 2015; Liang et al., 2016) and from 25 to 70 % (Ishfaq et al., 2020), respectively; the reduction in As grain content can be compensated by increases in cadmium (Cd) (Norton et al., 2017b), a health-hazardous heavy metal; whilst reductions in CH_4 can be partially or completely compensated by enhanced N_2O emissions resulting in an net increase of the global warming potential (GWP) (Lagomarsino et al., 2016; Kritee et al., 2018). The agronomic impact of AWD is also under debate with reported reduced (Linquist et al., 2015; Liang et al., 2016; Islam, 2018), unaffected yields (Carrijo et al., 2018; Runkle et al., 2019; Liao et al., 2020) or even enhanced rice production (Mofijul Islam et al., 2016) induced by soil aeration favouring root development (Zhang et al., 2009; Norton et al., 2017a)

The reasons of such a variability in the benefits/detriments of AWD are multiple but globally based on; firstly, the broad agronomic and environmental variability of rice agroecosystems with differing edaphic, climatic and agronomic conditions, and; secondly, the varying types of AWD regimes in terms of severity, *i.e.,* the critical threshold to which the water table is allowed to drop before reflooding (Linquist et al., 2015; Liang et al., 2016), and the moment of implementation (Boonjung and Fukai, 1996).

The varying outputs of AWD pose a limitation for its implementation so that a wide adoption of AWD in a rice growing area, wherein the pros are enhanced while the cons minimised, needs to be preceded by crop-context specific studies. AWD has been widely studied in Asian rice systems (Li and Barker, 2004; Lampayan et al., 2015; Mofijul Islam et al., 2016; Ishfaq et al., 2020) and in the USA (Linquist et al., 2015; LaHue et al., 2016; Carrijo et al., 2018). However, less is known about the potential benefits and trade-offs of AWD in Europe with the exception of Italy, where contrasting results on its GWP mitigation potential and yield impacts have been reported (Lagomarsino et al., 2016; Meijide et al., 2016; Monaco et al., 2021; Peyron et al., 2016; Oliver et al., 2019). To our knowledge, no studies have been conducted in Spain, despite being the second largest rice producing country in Europe.

Rice production in Spain accounts for 28% and 5% of the European production and crop extension, respectively. Rice in Spain is cultivated as a monocrop system with three periods with differing water managements: the growing (late April to September), post-harvest (October to December) and pre-growing (January to March) seasons. The standard water management during the growing season consists of fields permanently flooded to a depth of ca. 5 to 15 cm deep. In the post-harvest, the fields are left fallow and either maintained flooded or left to progressively drain, according to the either farmers' preferences or the agri-environmental schemes stablished in each growing region. Water supply is cut off in December hence the fields during the pre-growing season are dry in order to allow soil labouring. The irrigation water is derived from the river and supplied to rice fields through a huge canal network spread over the whole rice growing area.

A two-year field experiment was conducted in a representative Spanish rice growing area, the Ebre Delta. We tested the hypothesis that the benefits associated to AWD system, *i.e.,* mitigation of GHG emissions and reduction of grain element content, can be achieved within the context of a European rice crop system without compromising grain yield. In addition, the agronomic response to AWD of a set of representative European rice cultivars was assessed. This experiment was conducted in coordination to a parallel field study in Northern Italy (Monaco et al., 2021) with the overall goal of assessing the agronomic and environmental consequences of shifting the rice irrigation system from permanently flooded to AWD in rice cultivation in Europe.

2. MATERIAL AND METHODS

2.1 Site description

The study was conducted in 2016 and 2017 in the experimental rice fields of the Ebre Experimental Station of IRTA, located in the municipality of Amposta (40° 41’ 42’’N, 0 ° 47’ 00’’E) in the Ebre delta (Southern Catalonia, NE Spain). The climate of the region is Mediterranean with a mean annual precipitation of 500 mm, mostly distributed during spring and autumn. The mean annual air temperature is 18 ºC with mild winter (mean temperature in January 9 ºC) and summer (mean temperatures in July 24 ºC).

The soil texture (0 – 20 cm) of the field was silty clay loam (32.52 % clay; 64.5 % silt; 3 % sand) and the bulk density 1.3 g cm^{-3}. The pH was 8.3, and it contained 2.17 g kg^{-1} organic matter, 756 mg kg^{-1} of sulphates, 1.6 g kg^{-1} total nitrogen and 14.3 mg kg^{-1} Olsen phosphorous.

2.2 Crop management and experimental set-up

The experiment was laid out in a split-plot design, with four replicates. The main plots, of 250 m^2 each, represented the water management, including alternate wetting and drying (AWD) and permanent flooding (PFL), while the subplots, of 13.5 m^2 each, represented nine representative European rice cultivars from Spain: Gleva, Puntal and JSendra; Italy: Vialone Nano, Selenio and Loto; France: Arelate, Gines and, Gageron. The accessions were selected according to their representativeness and yield capacity, being all considered as high-yielding cultivars in their respective country. More information is provided in Supplementary table 1.

The AWD treatment was applied from the start of tillering until the start of flowering of the earliest variety to avoid spikelet sterility in any of the studied cultivars due to water-deficit stress imposed during the flowering (Lampayan et al 2015). This criterion was also followed by Norton et al. (2017a) and Carrijo et al., (2018). During the drying events, the water layer was left to progressively drop until a depth of approximately -20 cm (AWD threshold) and reflooded thereafter. Hydrological status of AWD plots was assessed by measuring both soil water potential with tensiometers (installed in all AWD plots at 25 cm depth) and water layer depth in piezometers (groundwater perforated PVC tubes of 150 cm long and 15 cm diameter, installed to a depth of 100 cm from the soil surface in all AWD plots).

Before and after AWD implementation, water management was the same in the two water treatments being the fields flooded at 5 to 10 cm deep and drained one month prior to harvest. In PFL, fields were permanently flooded at 5 to 10 cm deep throughout the growing season. The crop management was conducted following the standard practices of the area (Table 1). Soil labour operations were conducted in March in dry soil conditions, fields were flooded during the last week of April, and water seeded manually the first week of May. Each cultivar was independently harvested at the physiological maturity and when the grain moisture was around 20 – 21 %. The harvest was done over September and after that, the fields re-inundated before straw incorporation, in October, and left flooded until December.

2.3 GHG sampling and calculation of greenhouse emissions and global warming potential.

Closed opaque gas chambers (Altor and Mitsch, 2008) were used for gas sampling (more detailed description of the chambers and gas sampling procedure in Martínez-Eixarch et al., 2018). CH_4, CO_2 and N_2O were analysed simultaneously by a CG 7820A Agilent (USA) system equipped with a single channel and 2 valves of ten-port gas sampling with back-flush to vent and 6-port to change between the FID and micro-ECD detectors, using 2 packed columns Hayesep-Q 80-100 mesh 2 m x 1/8" × 2.0 mm Ultimetal Agilent (USA). Emission rates were calculated as the variation of gas concentration over the gas sampling in each chamber using linear interpolation. The increase of temperature in the headspace of the chamber was considered according to the ideal gas law. Only significant linear regressions ($p < 0.05$ and $R^2 > 0.80$) were accepted, and non-significant regressions were considered as zero emission rates. Cumulative GHG emissions between two consecutive sampling events were calculated assuming constant emission rates between them and then, they were all summed to calculate the seasonal cumulative CO_2, N_2O and CH_4 emissions. The overall global warming (GWP) effect , expressed in CO_2-equivalent units, was calculated considering a relative warming effect for CH_4 and N_2O 34 and 298 times higher, respectively, relative to CO_2 (Myhre et al., 2013).

Gas fluxes were only measured in Gleva cultivar subplots, in three out of the four replicates in each water treatment, and consistently within the same time window of 10.00 am to 1 p.m. During AWD implementation, gas sampling was conducted three times in each AWD cycle, that is at ca. -10 cm, -20 cm and +5cm water table depth, totalling 10 and 13 gas extractions over the 38 and 39-day period of AWD implementation in 2016 and 2017, respectively. Before and after AWD implementation, gas samples were collected on a bi-weekly and weekly basis, respectively. Simultaneously to gas sampling, soil parameters such as redox, electrical conductivity, temperature, and pH were measured next to the chambers at 10 cm soil depth.

2.4 Yield and yield-related traits

Grain yield and yield components per unit area were determined. Plant and panicle density per m^2 in each subplot were determined by counting panicle number in a 1-m^2 subarea (composite of four 0.25 m^2 subareas randomly placed in each subplot) at 4th leaf stage and heading. The remaining yield components, namely panicle size (spikelet number per panicle), spikelet fertility (filled grain number per panicle) and one-thousand grain weight, were calculated from 120 panicles randomly sampled in each plot. Separate grains in each plot were weighted and put in individual bags. Unfilled grains were separated by using a blower (Oregon Seed Blower) and then, spikelet fertility (number of filled grains per panicle) was calculated by dividing weight of filled grains by weight of total (filled and empty grains). Thousand-grain weight was calculated from the mean weight of six 500-grain samples whilst grain number per panicle from the average of panicle grain weight and the thousand-grain weight. Grain yield was determined from yield components. Plant height was measured in 4 randomly selected plants at the late milk phenological stage (77 BBCH). Indirect measurements of leaf nitrogen content were conducted using a chlorophyll meter, SPAD meter (Minolta Co.), with readings on 10 randomly assigned topmost fully expanded leaves (Cabangon et al., 2011).

2.5 Shoot and grain analyses

Fifteen productive tillers (stem + panicle) per subplot were randomly sampled at physiological maturity for determining grain and stem C and N content and grain heavy metal content, respectively. For the C and N content, dehusked rice grains and stems were oven dried (80 ºC), stems cut into 2-3 cm pieces and then a minimum of 0.3g of sample was randomly selected for ball milling. Milled powder of 0.006g was weighed into tin capsules. These samples were analysed on an Elemental Analyser (NA2500, Carlo Erba (CE) Instruments). Certified reference material (Beech Leaves [IRMM BCR®-100]) was used and repeated throughout the analysis as a quality control. For metalloids (As) and heavy metal content (Cd, Mn, Zn, Se and Cu), rice grains were dehusked and oven dried (80 ºC). For digestion, 0.2 g of dehusked grains were weighed out into 50 mL polyethylene centrifuge tubes. Grain samples were microwave digested (CEM Mars 5 microwave digester) with concentrated nitric acid and hydrogen peroxide as described in (Norton et al., 2012). Total element analysis (manganese, copper, zinc, arsenic, selenium, and cadmium) was performed by ICP-MS. Trace element grade reagents were used for all digests, and for quality control replicates of certified reference material (CRM) (Oriental basma tobacco leaves [INCT-OBTL-5], and rice flour [NIST 1568b]) were used; blanks were also included. All samples and standards contained 10 g L^{-1} indium as the internal standard.

2.6 Statistical analyses

A principal component analyses (PCA) was conducted to explore variability associated to year, water management and cultivar effect on yield, yield components and yield-related traits. To compare the agronomic, and As and heavy metal grain content response of the cultivars to water treatments, multivariate analyses of variance (MANOVA) was performed. MANOVA is used when several dependent variables are measured on each sampling unit instead of only one variable (for more details, see Rovira et al. (2012). Significances were further explored with three-way analysis of variance (ANOVA). Regarding the As and heavy metal grain content, when the concentrations were below the limit of detection (LOD), it was then assumed to be half of the LOD (Gu et al., 2020). The effect of water treatment of cumulative GHG emissions in Gleva cultivar was tested with ANOVA. Interannual variability was observed so that year was considered as a fixed factor in both MANOVA and ANOVA analyses. Statistical analyses were run with SPSS statistics software (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp).

3. RESULTS

The two-year mean temperature in winter (from December to February) and summer (from June to August) was 10.1 ± 0.5 ºC and 23.4 ± 0.4 ºC summer, respectively. Climatic conditions were similar over the two growing seasons, with mean temperatures and cumulative rainfall of 21.7 ± 1.4 ºC and 22.1 ± 1.2 and, 59.5 mm and 51.6 mm in 2016 and 2017, respectively.

3.1 Crop phenology and implementation of AWD and effects on soil redox

Tillering in Gleva started 45 and 36 days after sowing (DAS) in 2016 and 2017, respectively. The delay observed in the first year was probably caused by a post-herbicide shock which also provoked lower plant establishment, as later presented. Panicle initiation occurred 63 and 64 DAS, and heading, 88 and 83 DAS, in 2016 and 2017, respectively.

AWD was implemented from the start of tillering until flowering, *i.e.,* from 16th June to 29th July in 2016 and from 9th June to 27th July in 2017 (Fig. 1). Despite the targeted AWD threshold of – 20 cm, varying in-field AWD thresholds were finally registered (Table 2) mainly in terms of the soil water status readings.

Soil redox was increased by AWD but, regardless the water treatment, the ranges remained higher in 2016 (PFL: from - 194.9 ± 4.8 to + 66.5 ± 28.7 mV; AWD: from – 150.4 ± 13.6 to + 147.2 ± 0.0) than in 2017 (PFL: from – 258.4 ± 21.6 mV to – 146.3 ± 10.8; AWD: from – 188.0 ± 23.2 to – 12.0 ± 5.6). The critical range of soil redox values for methanogenic activity (– 100 mV to – 210 mV) was only achieved and established for a prolonged period in PFL fields in 2017.

3.2 Effect of AWD on canopy development, grain yield and yield-related traits

The crop establishment and crop growth in 2016 was significantly ($p < 0.05$) lower than in 2017 as indicated by the lower plant establishment (210.3 ± 8.2 *vs.* 252.9 ± 7.6 plants m^{-2}), plant height (84 ± 2 cm *vs.* 92 ± 2cm), canopy coverage (66 ± 9 % *vs*. 98 ± 2 %) and leaf N content at flowering (SPAD values: 38.5 ± 4.3 *vs.* 41.5 ± 35). The effect of water management on plant height and N leaf content across the cultivars and canopy development in Gleva (Fig. 2) was only significant in 2016: plant height ranged from 53 to 111 cm in AWD and from 63 to 122 in PFL, respectively, and the maximum canopy and SPAD values at flowering in PFL and AWD was 86 ± 6 *vs.* 47 ± 12 %, and 38.5 ± 4.3 *vs.* 37 ± 4 , respectively.

The PCA analysis conducted on yield and yield-related traits showed that most of the analysed variables were interdependent and significantly intercorrelated. The KMO measure of sampling adequacy (0.560) indicated the usefulness of the PCA, with the first two components explaining 41.2 % of the total variation (Fig. 3). The first PCA axis showed the associations between N content and yield-related traits. Nitrogen in shoots and grains at maturity were negatively correlated to N content in leaves at flowering (SPAD), C:N ratios in grain and shoots at maturity and, sink (spikelet number per m^2) and source (filled grain number per m^2) strength. The second PCA axis showed a negative correlation between yield and most of the yield components, and plant height and plant density. The distribution of the point scores in the PCA biplot presented two groups separated by the year: rice plants in 2016 showed lower leaf N content at flowering but large N content in shoot and grains at maturity than in 2017. The same is true for plants submitted to AWD in 2016 whereas no differentiated distribution along the PCA1 attributable to water management was observed in 2017. No specific distribution by cultivars along the two PCA axes was observed.

The multivariate analyses of variance (MANOVA) test showed an overall significant effect of water management (Wilks's $\lambda = 0.447$; $F_{7,21} = 17.85$; $p < 0.0001$), cultivar (Wilks's $\lambda = 0.001$; $F_{56,549} = 28.66$; $p < 0.0001$), year (Wilks's $\lambda = 0.304$; $F_{7,101} = 33.03$; $p < 0.0001$), and year × cultivar (Wilks's $\lambda = 0.227$; $F_{56,549} = 3.10$; $p < 0.0001$) , water management × year (Wilks's $\lambda = 0.551$; $F_{7,101} = 11.74$; $p < 0.0001$), water management × cultivar (Wilks's $\lambda = 0.316$; $F_{56,549} = 2.34$; $p < 0.0001$) and the water × cultivar x year interactions (Wilks's $\lambda = 0.415$; $F_{56,549} = 1.74$; $p < 0.001$) on yield and yield components.

Specifically, for yield and each yield-related trait, the results of MANOVA test as well as the mean marginal means are presented in Table 3. The overall mean annual grain yield in both years of the study was similar (chronologically: 7358 ± 207 *vs.* 7423 ± 211 kg ha^{-1}). Under PFL, grain yield in 2016 was slightly higher, but non-significantly ($p = 0.31$) than in 2017 (8106 ± 294.8 *vs.* 7577 ± 317 kg ha^{-1}) while the opposite was true in AWD(chronologically: 6590.0 ± 229.7 vs 7268.4 ± 282.1 kg ha^{-1}).

Averaged across the years and cultivars, AWD reduced grain yield 12 %; by years, the impact of AWD was larger and significant in 2016 (19 %) than in 2017 (6 %), being statistically insignificant.

The three-way effect of the studied factors, including year as fixed factor, revealed contrasting cultivar responsiveness to AWD in terms of both severity (magnitude) and direction over the two years of the study. The yield response to AWD across the cultivars is presented in Fig. 4. LOT was tolerant to AWD as shown by the similar grain yields in both water treatments over the two-year study. GAG, GIN, GLE, JSE consistently performed as sensitive cultivars to AWD with yield declines of 24 % (range: 20 % – 27 %) and 12 % (range: 10 % – 13 %) on average in 2016 and 2017, respectively, though the latter being non-significant. SEL showed similar yield loss under AWD than the sensitive group in 2016, *ca.* 24 %, but maintained the same yield in 2017. Finally, ARE, VIA, PUN showed contrasting interannual responses to AWD. VIA increased grain yield by 51 % under AWD in 2016 but then it declined by 8 % in 2017; ARE was affected by AWD by 23 % in 2016 but then, in 2017, it turned to perform 14 % better than PFL; PUN sharply declined grain yield by 37 % under AWD in 2016 but remained stable in 2017.

The response to AWD of yield components and yield-related traits were investigated to explore how AWD modulated yield formation (Table 3). Panicle density in LOT remained unaffected by AWD whereas it declined in GLE, GAG, GIN and JSE, by 6 – 7 %, and by 6 % and 21 % in SEL in 2016 and 2017, respectively. The number of spikelets per panicle (panicle size) was largely affected by AWD in 2016, with 21 % to 27 % in GLE, GAG, GIN, JSE, SEL and LOT, but to a lesser extent, 3 % to 4 %, in 2017. Sink size, which is the number of spikelets per m2, was reduced by 25 % in GAG, GIN, GLE, JSE and SEL in 2016 and by 12 % in LOT while in 2017 the reduction ranged from 4% to 6%. Rates of grain filling increased in 8 and 4 percentage points in LOT in 2016 and 2017, respectively, but in less than 3 percentage points in GLE, GAG, GIN, JSE and SEL in 2016 and 2017.

VIA, PUN, and ARE showed contrasting responses to AWD in the two years of the study. In 2016, PUN and ARE reduced panicle density (46 % and 15 %, respectively) and panicle size (33 % and 21 %, respectively) resulting in reduced sink size (48 % and 30 %, respectively). Thereafter, spikelet fertility was increased by ten percentage points in both of them. By contrast, none of the yield components was affected by AWD in 2017 in both cultivars. In 2016, VIA increased panicle density, panicle fertility and thousand-grain weight under AWD but reduced panicle size whereas in 2017, panicle size, panicle fertility and a thousand-grain weight were reduced while panicle density increased.

Regarding the overall cultivar performance, JSE consistently ranked among the best grain yielding cultivars within each year and water management, even with the yield declines suffered. The cultivars JSE, PUN, GLE and SEL, GAG showed the best performance consistently across the years and within each water management. By contrast, VIA showed the worst performance.

3.3 Effect of water management and cultivar on Arsenic and heavy metal content

The multivariate analyses of variance (MANOVA) test showed an overall significant effect of water management (Wilks's λ = 32.00; $F_{6,74} = 40.10$; $p < 0.0001$), cultivar (Wilks's λ = 4.83; $F_{48, 368} = 4.83$; $p < 0.0001$), year (Wilks's λ = 0.316; $F_{6,74} = 26.70$; $p < 0.0001$), year x water management (Wilks's λ = 0.70; $F_{6,74} = 5.28$; $p < 0.0001$) and water management x cultivar (Wilks's λ = 0.42; $F_{48,368} = 1.46$; $p < 0.05$) on the element concentration in the grains of rice. Marginal means (± SE) can be found in Supplementary table 2.

The grain concentration of As (Fig. 5, Table 4) ranged from 0.121 to 0.438 mg As kg^{-1}, being significantly ($p < 0.001$) larger in 2017 (0.28 ± 0.01 mg As kg^{-1}) than in 2016 (0.23 ± 0.01 mg As kg^{-1}). Globally, AWD decreased As concentration in all genotypes (Table 4, Fig. 5) by ca. 40 % in the two years (PFL vs AWD: 2016; 0.29 ± 0.01 *vs.* 0.17 ± 0.001; 2017, 0.35 ± 0.01 *vs.* 0.21 ± 0.01 mg As kg^{-1}.

Under PFL, all the cultivars surpassed the threshold of 0.20 mg As kg^{-1}, while the cultivars GAG and VIA (and LOT in 2017) consistently showed larger grain As than the remainder. Under AWD, the cultivars GIN, GLE, JSE, LOT and PUN maintained the grain As below 0.2 mg kg^{-1} consistently in both years of the study and GAG still showed the largest As concentration in both years.

The overall grain Cd concentration (Table 4, Fig. 5) was similar in the two years of the study (0.007 ± 0.001 mg Cd kg^{-1}) as well as the increase in AWD (PFL *vs.* AWD: 0.005 ± 0.001 *vs.* 0.009 ± 0.001 mg Cd kg^{-1}), though the concentration remained low in both treatments (< 0.018 mg Cd kg^{-1}). In 2016, Cd concentration in the cultivars ARE, GAG, PUN, SEL and VIA under AWD felt below the limit of detection (and so half of the sample LOD was used for statistical analyses, see Material and Methods). No specific cultivar pattern was observed for Cd concentration, safe LOT and VIA showing the largest concentrations under AWD (0.013 – 0.018 mg Cd kg^{-1}).

The concentration of Mn was not affected by the water management (24.93 ± 0.87 mg Mn kg^{-1}) whereas the genotype and genotype -by year effects were significant, being VIA, GLE and JSE the cultivars consistently showing the largest grain Mn concentration 29 – 41 mg Mn kg^{-1}) while LOT, GIN and ARE the lowest (<22 mg Mn kg^{-1}).

Cu, Se and Zn concentrations increased with AWD by 27 %, 78 % and 41 % respectively, with the water -by year effect significant which was explained by the consistent increase in both years but only significant in one of them. The cultivar effect was significant for Cu and Se.

3.4 Effects of AWD on GHG emission rates and cumulative GHG

CH_4 emissions in Gleva cultivar (Fig. 6) under the standard water management, *i.e.*, permanent flooding, were very low in 2016, showing a mean seasonal rate of 0.22 ± 0.12 mg CH_4 m^{-2} h^{-1} and totalling 1.85 ± 0.7 g CH_4 m^{-2} emitted over the growing season. In 2017, the mean seasonal rate of

CH_4 emissions under PFL was 2.45 ± 0.3 mg CH_4 m^{-2} h^{-1} totalling 7.0 ± 1.1 g CH_4 m^{-2} which was aligned with the emissions previously reported in the area by Martínez-Eixarch et al (2018, 2021). Similarly, more CO_2 was emitted in the second year of the study under the standard water management (Table 5) whereas negligible N_2O emissions were found in the two years (<0.01 g N2O m^{-2}).

AWD significantly reduced mean emission rates of CH_4 by 79 % and 94 % in comparison to PFL, in 2016 and 2017, respectively, leading to reduction in cumulative CH_4 emissions of 91 % to 95 % (Table 5). N_2O emissions rates (Supplementary Figure 1) were very low in the two water managements over the growing season though contrasting effects of AWD were found over the two years of the study: in 2016, mean emission rates were slightly reduced by 14 % in 2016, leading to 58 % less cumulative N_2O, but increased by 600 % in 2017, leading to 300 % more cumulative emissions (Table 5). N_2O fluxes were detected ten days after the fertilization events in AWD in 2016 ,10.5 ± 10.5 µg N_2O m^{-2} h^{-1}, and in both AWD and PFL in 2017, 2.5 ± 2.5 and 1.9 ± 1.3 µg m^{-2} h^{-1}, respectively. AWD significantly reduced mean emission rates of CO_2 by 58 % and 13 % in comparison to PFL, in 2016 and 2017, respectively (Supplementary Figure 1), leading to reduction in cumulative CO_2 emissions of 58 % and 44 % (Table 5) .

The resulting GWP (CH_4 + N_2O) was significantly reduced by AWD by 90 % and 96 %, in 2016 and 2017, respectively (Table 5). Therefore, the mitigation potential of AWD by reducing CH_4 emissions was not offset by enhanced N_2O emissions. CH_4 was the main contributor of GWP (> 90 %) in all the treatments.

4. DISCUSSION

4.1 Agronomic performance of the rice crop: interannual variability under permanently flooded fields

Plants grown in both water managements in 2016 presented symptoms of phytotoxicity caused by the application of the herbicide, namely reduced plant establishment, N content in leaves (SPAD), crop canopy and plant height (Jason et al., 2007; Awan et al., 2016). Plants under PFL could recover from the injury, as indicated by the comparable grain yield to that obtained in 2017. By contrast, the herbicide-induced impact apparently persisted and yet was aggravated by the implementation of AWD as indicated by the poor canopy development (Fig. 2).

4.2 Agronomic response to AWD

The present study examines the response of nine representative European cultivars submitted to AWD system. Overall, AWD implemented in 2016 was more severe than in 2017, since 7 out of 9 cultivars significantly reduced grain yield, whereas in the second year, only non-significant declining trend was observed in the cultivars JSE, GLE, GIG and GAG and VIA. The stronger severity of AWD in 2016 was likely explained firstly, by the weaker health conditions of the plants prior AWD implementation caused by the herbicide phytotoxicity and, secondly, by the excessive drought (– 25 ± 0 cm or – 54 ± 14 KPa) imposed around panicle initiation rather than by a repetitive exposure to water stress along the AWD cycles. It is then derived from this that the timing of AWD thresholds is decisive for yield response to water stress. Hereafter, AWD implemented in 2016 and 2017 will be referred as severe and mild AWD, respectively.

The present study revealed contrasting genotype response to AWD which is aligned with Bueno et al. (2010), and Liang et al. (2016) but contrasts with Zhang et al. (2009), Yang and Zhang (2010) and, Norton et al. (2017b). LOT was the only cultivar identified as tolerant to AWD, in agreement with Orasen et al. (2019) but in contrast with Miniotti et al. (2016). The group formed by JSE, GLE, GAG and GIN and SEL was sensitive to severe AWD whilst it showed a non-significant declining trend under mild AWD. ARE and PUN performed as highly sensitive to severe AWD, but tolerant to mild

AWD. Finally, VIA showed opposite responses in the two years of the study. The erratic response of these three cultivars (VIA, ARE and PUN) prevents drawing conclusions on their sensitivity to AWD.

Differential sensitivity of the cultivars to AWD during the growing stages across the cultivars was detected. Panicle size was consistently affected by severe AWD in all cultivars indicating that early reproductive stages are sensitive to AWD thresholds around – 25 cm or – 50 KPa but tolerant to – 19 KPa and – 20 cm. Similarly, Liang et al. (2016) found differing responsiveness of panicle size to contrasting severities of AWD. While severe AWD consistently affected panicle size, varying genotype responsiveness of panicle density severity was found. Reductions in panicle density can be given by either reduced tillering ability (Boonjung and Fukai, 1996; Martínez-Eixarch et al., 2015) and/or enhanced tiller abortion (Okada et al., 2002; Alou et al., 2018). In ARE and PUN, panicle exertion induced by water stress around panicle initiation in 2016 could have reduced panicle density (Okada et al., 2002). On the other hand, capacity is apparently resistant to AWD-induced stress, as indicated by the non- response in 2017, when soil during the vegetative stage was even drier than in 2016. Instead, the comparable reduction of panicle density of GLE, JSE, GAG and GIN over the two years suggests that tillering capacity, rather than either tiller abortion or constrained panicle exertion, was the main driver of this reduction. Contrasting with Zhang et al. (2009) and Norton et al. (2017), none of our studied cultivars seemed to present enhanced tillering ability under AWD.

Therefore, yield loss was explained by the cumulative effect of AWD on both panicle density and panicle size, that is sink size, and by the subsequent incapacity of the plants to sufficiently compensate such an impact by increasing grain filling rates. Indeed, this compensatory mechanism conferred the consistent tolerance to LOT under the two severities of AWD. Bueno et al. (2010) also pointed out strong compensatory mechanisms as the drivers of AWD tolerance in some cultivars.

VIA, which is a tall cultivar, was benefitted from severe AWD by reducing plant height (plant height in PFL and severe AWD: 103 ± 3 vs 118 ± 2 cm, data not shown) (Wang et al 2016) thereby conferring lodging resistance and, eventually favouring grain yield (Setter et al., 1997; Wang et al., 2012).

To summarize this agronomic section, severe AWD with drying events lower than – 50 KPa around early reproductive stages, cause substantial yield reductions. Yield penalties under severe AWD (< -50 KPa) have been reported elsewhere (Bueno et al., 2010; Yang and Zhang, 2010) but others found no effect (Carrijo *et al.* 2018). The milder version of AWD implemented in the second year of the study, consisting in keeping a critical mean AWD threshold of –25 KPa over the AWD cycles and, specifically of – 20 KPa around panicle initiation, does not have a significant impact on production. Despite this, some varieties showed a downward trend that we believe can be overcome with a critical threshold of –20 KPa overall the AWD implementation (Bouman et al., 2007). To achieve this, it is very important to have a good control of the soil hydrology as sudden drops in water potential can occur in a few days and have serious consequences for production. In addition, development of rice varieties adapted to AWD, that is with as high yields as the best high yielding variety under PFL, would contribute to this pursuit and to widening the adoption of AWD water management by farmers (Price et al., 2013; Volante et al., 2017). In our study, the AWD-reduced yields in the Spanish cultivars were on average larger than the remainder, likely because of their better adaptation to the local growing conditions.

4.3 AWD effects on concentration of Arsenic and heavy metal content in grain

The averaged As concentration in rice grains under permanent flooding conditions was 0.32 mg As kg^{-2}, which surpasses the FAO recommendation of 0.2 mg kg^{-1} (Codex Alimentarius Commission, 2014). This concentration is comparable to the levels reported in Arkansas (Norton et al., 2012;

Linquist et al., 2015) but lower than those in Texas (Norton et al., 2012) and higher than in Philippines (Islam et al., 2020) and California (Lahue et al., 2016).

Soil redox influences on As speciation and mobilisation in paddy soils thus mediating plant As uptake and subsequently the grain As concentration. Soils with low redox potential favour As solubility and thereby plant uptake, as reviewed by Meharg and Zhao (2012). Therefore, the more reducing conditions in the permanently flooded plots in 2017 explains the larger As content in grains.

Our study provides further evidence that the implementation of AWD significantly reduces As content in grain. In the case of Ebre Delta, 40 % of grain As reduction was achieved which allowed keeping the mean threshold below the upper limit recommended by FAO and the European Commission. This AWD-induced reduction is in accordance with other studies, although with wide overall variation, from 18 % to 63 % (Linquist et al., 2015; LaHue et al., 2016; Norton et al., 2017), attributable to genotype or site-specific rice cultivar physiology (Wu et al., 2011; Norton et al., 2012; Rai et al., 2015), timing and severity of AWD implementation (Linquist et al., 2015; Norton et al., 2017a; Carrijo et al., 2018; Carrijo et al., 2019), application of organic matter (Islam et al., 2020). The multifactorial nature of grain As response to water management highlights the need of site-specific studies to evaluate the potential of AWD to reduce grain As. Further, the present study reveals genetic variation in As concentration in rice grains across the most representative European cultivars, which is in line with previous research (Norton et al., 2009; Ahmed et al., 2011; Norton et al., 2017). In our field study, GAG consistently presented the highest As concentration under both water treatments in the two years whilst 5 out of the 9 cultivars (GIN, GLE, JSE, LOT and PUN) kept As concentration below the recommended threshold under AWD. The genotype effect can be driven by both the differing root anatomy modulating root radial oxygen loss (ROL) and iron plaque formation, which is related to As speciation and thus, As bioavailability (Mei *et al.*, 2012), and by differing abilities on uptake and/or partitioning As to grains (Suriyagoda et al., 2018). In addition, these processes can also be modulated by environmental and agronomic factors so that a genotype-by- environmental interaction, found in other studies (Norton 2009; Norton et al., 2010; Pillai 2010; Ahmed 2011) could be expected.

In contraposition to As, grain Cd increased with AWD by 80 % 2017, but remained below the maximum levels of 0.2 mg kg^{-1} determined by the European Commission (Commission Regulation (EC) No 629/2008) in both AWD and PFL. The levels found in our study fall within the range previously reported in Ebre Delta (Roig et al., unpublished) and in Bangladesh (Norton et al., 2017a; Norton et al., 2017b). The mobility and availability of Cd in paddy fields is related to soil redox potential so that the reducing environment promotes the reduction of sulfates to sulfides to which Cd^{2+} ions are bound thereby reducing its bioavailability (Rinklebe *et al.*, 2016). The formation of sulfides in our fields under the lowest the soil redox potential is assumed and supported by the soil sulphate content of 756 mg kg^{-1}. The insignificant effect of water management in 2016 could be explained by the number of cultivars showing Cd concentrations below the limit of detection. Despite the consistent overall trend of increasing Cd content across the cultivars, such an effect was only significant in some cultivars: ARE in 2016 and 2017 and, ARE, LOT and VIA in 2017.

No effect of water on Mn was detected, which is in contrast with Norton et al. (2017a) who found increased grain Mn under AWD. The stability of the Mn in soils is regulated by both redox potential and pH (Reddy and DeLaune, 2008). In alkaline soils the solubility of Mn is low because it can either coprecipitate with carbonates forming solid $MnCO_3$ under reducing conditions or remain present as solid Mn (IV) in the form of MnO_2 under oxidizing conditions (Pan et al., 2014). Therefore, the basic soil of our field experiments (pH=8.3), in contraposition with the acid soils in Norton's study, could explain the contrasting responsiveness of grain Mn content to water management. The significant genotype effect was also found by Norton et al. (2017b).

The grain Cu concentration is comparable to a previous research in Ebre Delta (Roig et al., unpublished). The increasing effect of AWD on Cu and Se is in agreement with Norton et al. (2017a, 2017b) and Zhou et al. (2018), respectively, while that on Zn agrees with Wang et al. (2014) but not with Norton et al. (2017b) who found no AWD response. The AWD-induced increase in Cu, Se and Zn deserves further research as they all are important elements for plant health and human nutrition (White and Broadley, 2009; White and Brown, 2010)

4.4 Greenhouse gas emission and GWP

The plausible herbicide phytotoxicity shown in the first year of the experiment, influenced both rice physiology and the system of plant-soil-atmosphere gas exchange that led to reductions in CH_4 and CO_2 emissions in Gleva.

Reduced CO_2 emissions in 2016 likely resulted from reduced foliar coverage, thus reducing area for gas exchange, leaf N content (Reich et al., 2008) and presumably stomatal conductance caused by N shortage (Hirasawa et al., 2010). They also indicate a net reduction of the ecosystem respiration, including heterotrophic and autotrophic respiration. Oliver et al. (2019) found, in a parallel field experiment in Italy, that ecosystem respiration was dominated by autotrophic respiration in relation to heterotrophic respiration, so that changes in the foliar coverage could have been the main driver of reduced CO_2 emissions. Nevertheless, reduced heterotrophic respiration could have also resulted from inhibited plant growth and rhizoexudate release caused by N shortage thereby limiting carbon substrate for soil microorganisms.

The emissions of CH_4 in 2016 under the standard water managements were unexpectedly lower than those previously reported in rice fields in Ebro Delta (Martínez-Eixarch et al., 2018; Martínez-Eixarch et al., 2021). The low emissions are coherent with the interval of soil redox potential recorded throughout the growing season (–194.9 ± 4.8 to + 66.5 ± 28.7 mV) remaining mostly outside of the critical threshold for methanogenesis (Eh< – 150 mV; (Hou et al., 2000); Sahrawat 2005). Three processes mediated by the development of canopy and its positive association with the vegetative growth (Dingkuhn et al., 1999, 2000) could explain reduced CH_4 emissions. The first is a limited transport of CH_4 from the soil to the atmosphere via rice stems, since plant mediated transport is the major pathway of CH_4 diffusion (Aulakh et al., 2000; Pittelkow et al., 2013). The second, low crop development indicate low release of rhizoexudates and so reduced availability of labile organic matter, which is the predominant source for methanogenesis via DOC (Aulakh et al., 2001; Kögel-Knabner et al., 2010). The third, is that low N availability limits methanogenesis as supported by the positive association between rates of N fertilization rates and CH_4 emissions in Ebro delta rice fields (Martínez-Eixarch et al., 2021) and the conclusions extracted from meta-analyses by Liu and Greaver(2009) and Banger et al. (2012).

Our study provided further evidence that AWD can significantly reduce both CH_4 and GWP of paddy rice which is aligned with past studies (Linquist et al., 2015; Lahue et al., 2016; Runkle et al., 2019; Islam et al., 2020; Wang, 2020) but in contrast with others reporting increases of net GWP driven by increased N_2O emissions during the aeration events eventually offsetting CH_4 mitigation (Lagomarsino, 2016; Krittie, 2018; Liao et al., 2020;). In our study, even the increased N_2O found in 2017 could not compensate the decline in CH_4. No fluxes of N_2O during the aeration events were observed, only seven days after the fertilization in 2016 and with low values, suggesting that the nitrification process was likely completed to N_2 production, and that an appropriate N management was implemented, consisting in reflooding the fields the day after the topdressing N application since N_2O peaks in intermittent irrigation are linked to N fertilization events (Peyron et al., 2016; Lagomarsino et al., 2016). Further, the range of redox potential found in AWD fields mostly felt within the optimum window of soil redox potential (from +120 to –170 mV) in paddy soils around pH neutrality, in which both CH_4 and N_2O emissions are minimized (Yu et al., 2001; Yu and Patrick, 2003). This could also explain the large CH_4 mitigation capacity of AWD in our studied conditions, around 90 %, which is comparable to that reported by Linquist et al. (2015) and Peyron et al. (2016). Another plausible explanation for such a large CH_4 mitigation capacity would be that CH_4 fluxes did not completely recover after AWD cycles in relation to those in PFL, as also observed by Linquist et al. (2015), suggesting a lag phase for methanogenesis after the aeration periods. However, it should be noted that the low frequency of GHG sampling from the last AWD cycle to the maturity could have also underestimated CH_4 emissions during this period. More frequent sampling beyond the period of AWD implementation is necessary to precisely estimate its mitigation capacity.

5. CONCLUSIONS

This study confirms that AWD can be safely implemented with limited or null yield impact while obtaining the associated environmental benefits, namely, reduced CH_4 emissions and As content in grain. The safe version of AWD consists in implementing this water management from tillering to flowering and keeping the critical AWD threshold at –20 cm, with special attention around panicle initiation, which was the most sensitive growing stage.

The cultivar effect on AWD response was significant. The cultivar LOT was tolerant to AWD, even to a severe version of AWD, being such a tolerance conferred by the strong capacity to compensate reduced panicle size by enhancing grain filling rates. The slight non-significant yield decline found in the group formed by GLE, JSE, GAG and GIN under mild AWD can likely be overcome by a precise implementation of safe AWD.

AWD significantly reduces CH_4 emissions and the GWP by up to 90% being the large mitigation capacity explained by the negligible N_2O emissions found in both water treatments.

Our study provides further evidence that the implementation of AWD significantly reduces As content in grain, by 40 % in the case of Ebre Delta rice growing conditions, thus allowing to keep the mean threshold below the upper limit recommended by the European Commission. By contrast, Cd increases by 20 % with AWD, though the concentration remained below the maximum allowed levels.

The present is the first comprehensive study of the implementation of the AWD irrigation system in a Europe rice growing system in which climate change mitigation (GHG emissions), food safety (metalloid content in grain) and agronomic (yield) factors have been examined under an integrative approach. Moreover, the analyses of the genotype x management interaction, based on a set of the 9 most representative European cultivars, provides new knowledge transferrable either to breeding programs so that it can be exploited to better utilize European collection of germplasm, or to the rice farm sector to facilitate a safely implementation of this system avoiding risks of yield reduction. Altogether represents a step forward towards to the implementation of a more sustainable economically and environmentally rice cultivation in Europe.

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CAPTIONS

Table 1. Crop management in experimental fields in the two years of the study (2016 – 2017)

Table 2. Minimum water table depth and soil water status (AWD thresholds) achieved at each wetting and drying cycle over AWD implementation in 2016 and 2017. Data represent mean across the four replicates ± SE.

Table 3. Marginal means (± standard error) of yield, and yield-related traits (above) in both water treatments, AWD and PFL, in the nine European rice cultivars in the two years of the study (2016 – 2017) and (below) MANOVA of the effect of water management (WM), cultivar (CV) and year (Y) and their interactions on yield and yield-related traits. The asterisks indicate significance of the factor at 0.05 (*), 0.01(**) and 0.001 (***) levels. Abbreviations. Rice genotypes: ARE, Arelate; GLE, Gleva; PUN, Puntal; GAG, Gageron; JSE, JSendra; SEL, Selenio; GIN, Gines; LOT, Loto; VIA, Vialone Nano. Yield and yield-related traits: GYL, grain yield (kg ha^{-1}); PND, panicle density; TGN, total number of spikelets per panicle; TGW, one thousand-grain weight (g); FER, panicle fertility (%filled grains per panicle); SINK, sink strength (grain number per m^2).

Table 4. MANOVA of the effect of water management, cultivar and year and their interactions on the grain element concentration. Abbreviations: Y, year; WM, water management; CV, cultivar. The asterisks indicate significance of the factor at 0.05 (*), 0.01(**) and 0.001 (***) levels.

Table 5. Marginal means (± standard error) of the seasonal CH_4, N_2O, CO_2 and GWP in both water treatments, AWD and PFL, in Gleva cultivar in the two years of the study (2016 – 2017) and ANOVA of the effect of water management (WM), year (Y) and their interaction (WM x Y). The asterisks indicate significance of the factor at 0.05 (*), 0.01(**) and 0.001 (***) levels.

Table S1 Main agronomic traits of the studied cultivars.

Table S2 Marginal means (± standard error) As and heavy metal concentration in both water treatments, AWD and PFL, in the nine European rice cultivars in the two years of the study (2016 – 2017)

Figure 1. Water regime of experimental rice plots under AWD and PFL in the two years of the study (2016 and 2017). Arrows indicate the date of panicle initiation (chronologically: 63 and 64 days after DAS) and flowering (chronologically: 88 and 83 DAS) in Gleva cultivar. AWD was implemented from 16th June 2016 (45 DAS) to 29th July 2016 (88 DAS) and from 9th June 2017 (36 DAS) to 27th July 2017 (84 DAS).

Figure 2. Canopy development in Gleva cultivar over the rice-growing period in AWD and PFL treatments in the two years of the study (2016 – 2017).

Figure 3. Principal component analyses (PCA) of grain yield and yield related traits. A) Factor loadings of the variables and, B) samples scores showing the distribution of water management, AWD (solid circles) and PFL (empty circles), and the cultivars. Abbreviations. Rice cultivars: ARE, Arelate; GLE, Gleva; PUN, Puntal; GAG, Gageron; JSE, JSendra; SEL, Selenio; GIN, Gines; LOT, Loto; VIA, Vialone Nano. Agronomic traits: Grain_N, grain N content; Shoot_C, shoot C content; Shoot_N, shoot N content; Grain_C, grain C content; PN_PL, panicle number/plant; FER, panicle fertility; PND, panicle density; StH, time from sowing to heading; TGW, thousand-grain weight; HGT, plant height; PLD, plant density; GYL, grain yield; SINK strength (grain number m^{-2}); SOURCE strength (filled grain m^{-2}); GrainCN, grain C:N; ShootCN, shoot C:N ratio; SPAD at flowering.

Figure 4. Marginal means (± standard error) of yield in each cultivar under AWD and PFL in the two years of the study (2016 and 2017). Error bars indicate the standard error of the marginal mean. Abbreviations. Rice genotypes: ARE, Arelate; GLE, Gleva; PUN, Puntal; GAG, Gageron; JSE, JSendra;

SEL, Selenio; GIN, Gines; LOT, Loto; VIA, Vialone Nano. The rectangles indicate the cultivars with consistent interannual pattern across the years: the tolerant cultivar LOT and the sensitive cultivars JSE, GLE, GIN, GAG.

Figure 5. Marginal means (± standard error) of grain concentration of As (above) and Cd (below) under the studied water treatments, AWD and PFL, in nine representative European rice cultivars, in the two years of the study (2016 – 2017). Abbreviations. Rice cultivars: ARE, Arelate; GLE, Gleva; PUN, Puntal; GAG, Gageron; JSE, JSendra; SEL, Selenio; GIN, Gines; LOT, Loto; VIA, Vialone Nano. Note that the order of the cultivars in the X-axis and the scale in the Y-axis are different for As and Cd figures.

Figure 6. Mean emission rate of CH_4 (mg m^{-2} h^{-1}) over the growing in the two studied water treatments, AWD and PFL, over the two years of the study (2016 – 2017). The bars represent the standard error of the mean.

Figure S1 Mean emission rate of (above) N_2O (µg m^{-2} h^{-1}) and (below) CO_2 (mg m^{-2} h^{-1}) over the growing in the two studied water treatments, AWD and PFL, over the two years of the study (2016 – 2017). The bars represent the standard error of the mean.