1	The study site determines the magnitude of N2O emissions from Southern German
2	vegetable production but not the effectiveness of mitigation strategies
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7	Supplement tot he poster contribution of the N workshop, Aarhus, June 2024
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### 10 Material and Methods

## 11 Study sites

The two field experiments were established in two vegetable production areas in Southern 12 Germany. One study site was one of the experimental farms of the University of Hohenheim 13 ("Heidfeldhof") located 13 km south of Stuttgart (48° 43' 00" N; 9° 11' 40" E). Soil type was 14 a Haplic Luvisol derived from periglacial loess. The farm is located 410 m above sea level. The 15 second study site was the experimental farm of the LUFA Speyer ("Rinkenbergerhof") located 16 4 km north of Speyer (49° 21' 21" N; 8° 24' 57" E). Soil type was sandy Cambisol derived 17 from sandy sediments of the Rhine river. The field experiment was located 99 m above sea 18 19 level. The distance between both study sites was approximately 130 km. The most important 20 soil chemical and physical characteristics of the study sites are shown in table 1.

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**Tab. 1:** Soil (0-0.3 m depth) and site characteristics from the two study sites in South Germany.

	Soil texture			Corg	Nt	pH*	MAT	MAP
	Sand	Silt	Clay					
	[%]	[%]	[%]	[%]	[%]		[°C]	[mm a <sup>-1</sup> ]
Hohenheim	2	68	30	1.80	0.16	6.5	9.9	686
Speyer	80	14	6	0.72	0.07	6.2	10.0	593

\* determined in 10<sup>-2</sup> *M* CaCl<sub>2</sub> solution, MAT: long-term mean annual air temperature; MAP: long-term mean annual

24 precipitation

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### 26 Field experiments

The field experiments were conducted at both sites simultaneously between August 2011 and March 2013 (20 months in total). In 2011 cauliflower (*brassica oleracea var. botrytis*, variety Fortaleza; 30,000 plants ha<sup>-1</sup>) was planted in late summer/autum 2011 and iceberg lettuce (*lactuca sativa var. capitata*, variety Diamantinas; 110,000 plants ha<sup>-1</sup>) followed by broccoli (*brassica oleracea var. italica*, variety Parthenon; 38,000 plants ha<sup>-1</sup>) were planted in 2012. Experimental set-up was a randomized completed block design with four replicates. Randomization was done for each site separately. Plot size was 4.5 m x 6 m (27 m<sup>2</sup>). Field management was handled according to conventional farmer's practice. Plant protection
measures were applied site specific. Planting was conducted for each of the three vegetable
crops simultaneously at both sites. Irrigation was carried out according to the irrigation tool
"agrowetter" provided by the German Meteorological Service (DWD).

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# 39 Experimental treatments

At each site, five different treatments were tested: i) unfertilized control treatment (control), ii) 40 treatment with high N-fertilization (high N) where we assumed that farmers apply N with safty 41 margins and without taking the initial mineral N into account for their calculation of N-fertilizer 42 43 demand (basis of the N-fertilization assessment in this treatment was also the result of personal communications of official consultants in vegetable production as the upper "N range" applied 44 in practice), iii) N-fertilization according to the German target value and consulting system 45 46 "kulturbegleitendes N<sub>min</sub> Sollwertsystem" (Feller et al. 2007) (opt N), iv) N-fertilizer amount calculated according to the "opt N" system and application in a single dose together with the 47 nitrification inhibitor 3,4 dimethylpyrazole phosphate (+NI), and v) N-fertilzer amount 48 calculated according to "opt N" with continious removal of all above ground crop residues after 49 harvest (-CR). 50

51 Ammonium sulfate nitrate (ASN, total N 26 %, NH<sub>4</sub>-N 19 %, NO<sub>3</sub>-N 7 %) was used for all Nfertilization measures (also in the +NI treatment). Treatment +NI was generally fertilized with 52 a single N dose whereas the other treatments received two N doses (Table 2). In the treatments 53 54 iii) -v) total amount of N fertilized to the single crops varied slightly between the study sites due to different initial mineral N contents being used for the calculation of N fertilizater 55 applications. In case mineral N before first N application was as high or higher as the amount 56 covering plant demand, we renounced on the first mineral N dose. At site Hohenheim, treatment 57 -CR in cauliflower in 2011 unintentionally received too little N. 58

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**Tab. 2:** Amount of N-fertilizer applied to the vegetable crops [kg N ha<sup>-1</sup>] in the different treatments between August 2011 and September 2012. Opt N = fertilized according to the German target value system "kulturbegleitendes N<sub>min</sub>-Sollwertsystem", NI = nitrification inhibitor, -CR = removal of the aboveground crop residues.

Treatment	Study site	Cauliflower	Lettuce	Broccoli
Control	Hohenheim	0	0	0
	Speyer	0	0	0
High N	Hohenheim	130+195	170+0	140+170
	Speyer	130+195	170+0	140+170
Opt N	Hohenheim	100+150	0+85	10+170
	Speyer	125+150	0+75	0+190
+NI	Hohenheim	240+0	85+0	180+0
	Speyer	275+0	75+0	190+0
-CR	Hohenheim	100+20*	20+85	30+145
	Speyer	125+150	20+65	50+200

<sup>64</sup> 

\* -CR treatment in cauliflower 2011 was unintentionally fertilized too low at study site Hohenheim.

### 66 Flux rate determination

67 Flux rate measurements were conducted at least once a week in the morning using the closedchamber method (Hutchinson and Mosier, 1981). A closer description of the circular dark, 68 vented PVC chambers was given in detail by Flessa et al. (1995). Additional gas samplings 69 were performed when high N<sub>2</sub>O fluxes were expected (after heavy rain fall, after N applications 70 during the growing season and during thawing of frozen soil in winter). When compared to 71 continuous measurements, a strict weekly gas sampling scheme can lead to an error of the 72 73 cumulative N<sub>2</sub>O emission in the range of  $\pm 20$  % (Parkin, 2008), whereas the additional eventoriented sampling can reduce this error below 10 % (Flessa et al., 2002). 74

The PVC chambers (0.15 m height) with inner diameter of 0.3 m were closed for 45 minutes. During this time, we periodically took four gas samples out of the chamber's atmosphere with a syringe through a sampling port and immediatedly transferred the samples into evacuated glass vials (22.4 ml) crimped with butyl septa. N<sub>2</sub>O and CO<sub>2</sub> concentrations in the gas samples were measured using a gas chromatograph (GC) equipped with a <sup>63</sup>Ni electron capture detector (ECD) (5890 series II, Hewlett Packard) coupled with an autosampler (HS 40, Perkin Elmer).

<sup>65</sup> 

GC configuration with a backflush of water vapour was adjusted in accordance to Loftfield et
al. (1997). Gas flux rates were calculated using the linear slope of the trace gas concentrations
in the chambers over time as described by Ruser et al. (1998).

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85 Soil and plant sampling and laboratory analyses

Simultaneousely to nearly all trace gas samplings, soil samples were taken from a depth of 0-0.3 m with a soil auger (0.014 m inner diameter). In order to reduce laborious soil analysis, we homogenized three single samples from each plot over the four replicates resuling in one composite soil sample per treatment and sampling date.

90 Soil samples were transported cooled to the lab and stored frozen until extraction for mineral 91 N. Before extraction, frozen samples were thawed overnight in a refrigerator. 40 g of fresh soil were extracted with 80 ml of 0.5 M potassium sulfate solution for one hour. Concentrations of 92 93 NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> were determined photometrically using a flow injection analyzer (3 QuAAtro, SEAL Analytical, UK). Soil water contents were determined gravimetrically after drying a 94 subsample at 105 °C over night. Once during the growing period of each vegetable crop and 95 once in winter we also determined bulk density of the A<sub>p</sub> horizon using stainless cylinders with 96 100 ml volume (10 random replicates). We used the mean bulk density of these values in each 97 98 year to calculate water-filled porosity (WFPS) as described by Guzman-Bustamante et al. (2019). 99

At the same time of gas sampling, we also determined soil temperature in 0.05 m depth. Air temperature (2 m height) and daily precipitation rates (mm) were provided by weather stations near to the experimental fields (0.5 km away from the site Hohenheim and 1 km away from the site Speyer).

To determine above ground N uptake, plants were harvested when the majority (>90%) of the plants in the fertilized treatments was in full marketable stage. From each plot centre 25 plants were cut manually. Total plant weights and marketable parts were determined for the 107 calculation of above ground biomass and marketable yield. Aliquot samples were dried at 60
108 °C for 48 h to determine dry matter yield. Dried samples were ground and C and N
109 concentrations were analyzed using an elemental analyzer (vario MAX CN, Elementar
110 Analysensysteme, Hanau, Germany).

- 111
- 112 Further calculations and statistical analyses
- 113 *Cumulative N<sub>2</sub>O emissions and calculation periods*

Cumulative N<sub>2</sub>O emissions were calculated stepwise, assuming a constant flux rate until next sampling date (Guzman-Bustamante et al., 2019). Cumulative emissions were calculated separately for the following time periods: cauliflower (01.08.2011 – 21.11.2011), late autumn 2011 and winter 2011/12 in the following referred to as "winter 1" (22.11.2011 – 24.04.2012), lettuce & broccoli (25.04.2012 – 18.10.2012), late autumn and winter 2012/13 in the following referred to as "winter 2" (19.10.2012 – 31.03.2013), and for the total experimental period (01.08.2011 – 31.03.2013).

121

122 Weather conditions during the experiment

Overall, air temperature during the different experimental periods was between 1 to 2°C higher 123 124 in Speyer than in Hohenheim (Table 3). Figure 1 shows daily precipitation rates and daily average air temperatures (2 m heigth). Calculation of a linear regession between air 125 temperatures at the two study sites revealed a 1.15°C lower temperature in Hohenheim 126 (intercept value) whereas the slope of the regression was .998, indicating almost identical 127 temperature dynamics at the two study sites ( $r^2=0.98$ ; data not shown). Due to the higher air 128 temperature, harvest of the vegetable crops in Speyer was frequently earlier than in Hohenheim. 129 Initially, late summer and autumn in 2011 were relatively warm followed by a cold winter 130 2011/12 with severe frost events (Figure 1) with soil being frozen down to 0.3 m depth in 131 132 Hohenheim.



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Fig. 1: Water-filled pore space (WFPS) (mean over all treatments), daily precipitation,
irrigation and mean daily air temperature (2 m height) at the two study sites.

Rainfall during the cauliflower period in 2011 was nearly the same for both study sites (145 mm in Hohenheim and 159 mm in Speyer, table 3). However, due to the low water-holding capacity and probably also due to higher evapotranspiration as a result of the higher temperatures, 125 mm were additionally irrigated in Speyer whereas in Hohenheim only 34 mm were irrigated in 2011. In total Speyer received 105 mm more rainfall and irrigation than Hohenheim in this period. Similarly, rainfall during the cropping season 2012 was 307 mm in Hohenheim and 345 mm in Speyer. In this period, Hohenheim received 56 mm irrigation and

143	Speyer was irrigated with additional 329 mm. Over the whole experimental season, total
144	precipitation (including irrigation) was 1041 mm in Hohenheim and 1392 mm in Speyer.
145	Despite higher rainfall and irrigation at the study site Speyer, the water-filled pore space
146	(WFPS) was higher at Hohenheim. The mean soil moisture over the entire measurements was
147	39.8% WFPS in Speyer and 57.3% WFPS in Hohenheim (mean over all treatments) (figure 1).
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**Tab. 3:** Precipitation (Prec), irrigation (Irr) and mean air temperature in 2 m height as affected
by study site and experimental period.

Period	Study site	Precipitation	Irrigation	$\Sigma$ Prec & Irr	Temp
	-	[mm]	[mm]	[mm]	[°C]
Cauliflower	Hohenheim	145	34	179	13.4
	Speyer	159	125	284	14.4
Winter 1	Hohenheim	212	-	212	3.8
	Speyer	191	-	191	5.1
Lettuce &	Hohenheim	307	56	363	16.5
broccoli	Speyer	345	329	673	17.7
Winter 2	Hohenheim	288	_	288	2.6
	Speyer	243	-	243	3.8
Total	Hohenheim	952	90	1041	9.1
	Speyer	939	454	1392	10.2

151 Cauliflower: 01.08.2011 – 21.11.2011; winter 1: 22.11.2011 – 24.04.2012; Lettuce & broccoli: 25.04.2012 –

**152** 18.10.2012; winter 2 (19.10.2012 – 31.03.2013); total (01.08.2011 – 31.03.2013).

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