

Effect of Climatic Conditions, and Agronomic Practices used in Organic and Conventional Crop Production on Yield and Nutritional Composition Parameters in Potato, Cabbage, Lettuce and Onion; Results from the long-term NFSC-Trials.

Leonidas Rempelos^{1,2}, Marcin Barański^{2,3}, Enas Khalid Sufar², Jenny Gilroy², Peter Shotton², Halima Leifert², Dominika Średnicka-Tober^{2,4}, Gultekin Hasanaliyeva⁵, Eduardo A.S. Rosa⁶, Jana Hajslova⁷, Vera Schulzova⁷, Ismail Cakmak⁸, Levent Ozturk⁸, Kirsten Brandt⁹, Chris Seal⁹, Juan Wang^{2,10}, Christoph Schmidt^{2,11} and Carlo Leifert^{12,13,*}

¹ Lincoln Institute for Agri-Food Technology, University of Lincoln, Riseholme Park, Lincoln LN2 2LG, UK

² Nafferton Ecological Farming Group, School of Agriculture, Food and Rural Development, Newcastle University, Newcastle upon Tyne, Tyne and Wear, NE1 7RU, UK

³ Laboratory of Neurobiology, Nencki Institute of Experimental Biology, Polish Academy of Sciences, Pasteura 3, Warsaw 02-093, Poland

⁴ Institute of Human Nutrition Sciences, Warsaw University of Life Sciences, Nowoursynowska 159c, 02-776 Warsaw, Poland

⁵ School of Animal, Rural and Environmental Sciences, Brackenhurst Campus, Nottingham Trent University, Nottinghamshire NG25 0QF, UK

⁶ Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), University of Trás-os-Montes and Alto Douro (UTAD), 5001-801 Vila Real, Portugal

⁷ Department of Food Analysis and Nutrition, University of Chemical Technology (UCT), Prague, 166 28 Prague, Czech Republic

⁸ Faculty of Engineering and Natural Sciences, Sabanci University, 34956 Istanbul, Turkey

⁹ Human Nutrition and Exercise Research Centre, Population Health Sciences Institute, Newcastle University, Newcastle upon Tyne NE2 4HH, UK

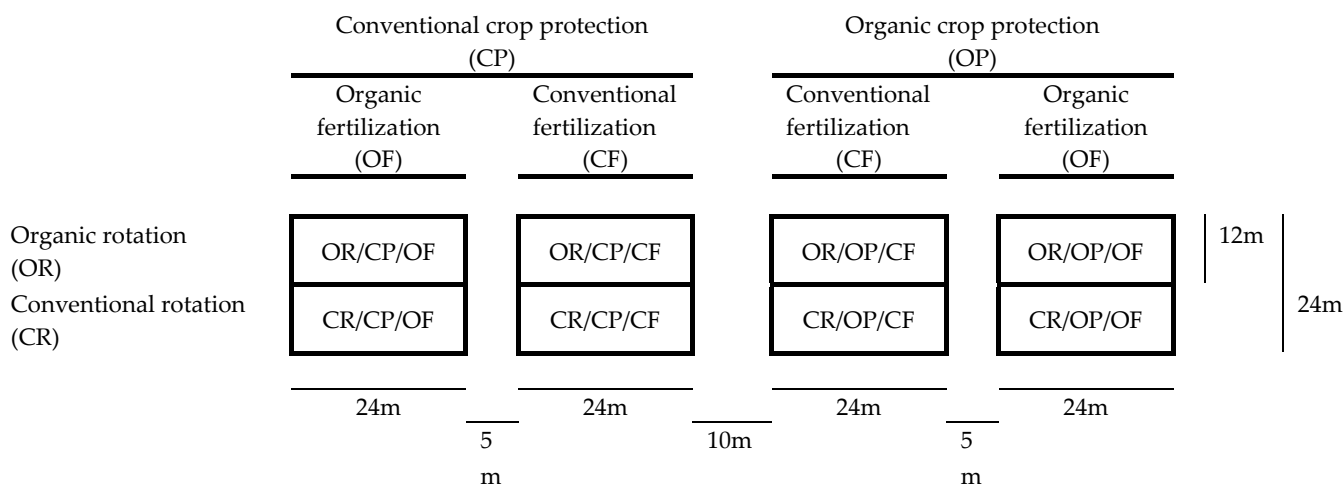
¹⁰ School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai 200240, China

¹¹ Institut für Analytik und Umweltchemie, Thomas-Mann-Straße 2, 98724 Neuhaus am Rennweg, Germany

¹² Department of Nutrition, Institute of Basic Medical Sciences, University of Oslo, 0372 Oslo, Norway

¹³ SCU Plant Science, Southern Cross University, Military Rd., Lismore, NSW 2480, Australia

* Correspondence: carlo.leifert@gmail.com



Supplementary Figure S1. Principal layout of rotation main plots, crop protection subplots and fertilization subplots in each of the four replicate experiments.

Supplementary Table S1. Climatic conditions during the three growing seasons in which phytochemical analyses were carried out.

Year	Parameter	April	May	June	July	August	September
2005	Precipitation (mm)	92	28	55	70	26	54
	Mean Relative Humidity (%)	81	78	81	81	81	83
	Mean Air Temperature (°C)	7.3	9.6	13.7	14.6	14.5	13.5
	Mean Soil Temperature (°C)	8.0	10.7	14.6	16.2	15.6	14.4
2006	Precipitation (mm)	22	74	28	13	68	71
	Mean Relative Humidity (%)	70	76	75	70	79	81
	Mean Air Temperature (°C)	6.9	9.7	14.0	17.4	14.4	14.8
	Mean Soil Temperature (°C)	7.5	10.8	15.4	19.6	16.2	15.1
2007	Precipitation (mm)	13	51	118	69	36	23
	Mean Relative Humidity (%)	77	77	83	80	78	78
	Mean Air Temperature (°C)	9.3	10.0	12.9	13.9	14.0	12.3
	Mean Soil Temperature (°C)	10.5	12.9	14.9	15.7	15.5	13.3

Supplementary Table S2. Rotation/sequence of crops in the four replicate experiments of the Nafferton Factorial Systems Comparison (NFSC) trial between the start of the experiment in 2001 and 2008.

Replicate Experiment	Rotation type	year							
		2001	2002	2003	2004	2005	2006	2007	2008
1	ORG	G/C	G/C	w-wheat	Veg	s-beans	Veg	s-barley	G/C
	CON	G/C	G/C	w-wheat	w-barley	w-barley	Veg	w-wheat	w-barley
2	ORG	G/C	G/C	GC	w-wheat	Veg	s-beans	Veg	s-barley
	CON	G/C	G/C	GC	w-wheat	w-wheat	w-barley	Veg	w-wheat
3	ORG	G/C	G/C	GC	Veg	G/C	G/C	w-wheat	Veg
	CON	G/C	G/C	GC	Veg	G	G	w-wheat	w-wheat
4	ORG	G/C	G/C	Veg	s-barley	G/C	G/C	G/C	w-wheat
	CON	G/C	G/C	Veg	w-wheat	w-barley	G/C	G/C	w-wheat

ORG, divers, legume-rich organic rotation; **CON**, non-divers, cereal-dominated conventional rotation; GC, grass clover ley; G, pure rye grass ley; w-wheat, winter wheat; w-barley, winter barley; s-barley, spring barley; s-beans, spring beans; Veg, potato in a 6×24 m sub-sub-subplot, and cabbage, lettuce, onion and carrot in 6×6 m sub-sub-subplots within each of the 12×24 m fertilization sub-subplots of the experiment.

Supplementary Table S3. Crop protection/defoliation protocols and fertilization regimes used in potato crops.

Fertilization regimes

Organic (OF) Composted cattle farmyard manure equivalent to 170 kg N/ha per year

Conventional (CF) Ammonium-nitrate (Nitram) equivalent to 180kg N/ha, and Superphosphate and KCl as a compound fertilizer (0:20:30) equivalent to 134kg P₂O₅/ha and 200 kg K₂O /ha

Crop protection protocols

Organic (OP)

Weed control 2 × ridging of potato rows per year

Pest control none

Disease control Copper Oxychloride; 5 applications per year

Defoliation 30/08/05 flailed 26/08/06 flailed 10/08/07 flailed

Conventional (CP)

Weed control linuron 1 application per year

Pest control aldicarb 1 application per year, no application in 2007

Disease control fluazinam 4 applications in 2005 and 2006, 5 applications in 2007

mancozeb and metalaxyl-M; 3 applications per year

Defoliation 30/08/2005 24/08/06 and 08/09/06 20/08/07 flailed and desiccated with diquat 28/08/07 desiccated with diquat

Supplementary Table S4. Crop protection protocols and fertilization regimes used in cabbage crops.

Fertilization**regimes**

Organic (OF) Composted cattle farmyard manure equivalent to 250 kg N/ha per year

Conventional (CF) Ammonium-nitrate (Nitram) equivalent to 260 kg N/ha and Superphosphate and KCl as a compound fertilizer (0:20:30) equivalent to 100kg P₂O₅/ha and 150 kg K₂O/ha

Crop protection**protocols****Organic (OP)**

Weed control 2 × hoeing with tractor and front-end hoe, 3 × manual hoeing

Pest control Capatex insect proof protective netting

Disease control none

Conventional (CP)

Weed control Propachlor: one application per year, rate 9 l/ha

Pest control Chlorpyrifos 2004 one application, 2005-2007 two applications, rate 1 l/ha,

Cypermethrin (Toppel 10): 2004-2006 one application, rate 250 ml /ha

Disease control Chlorothalonil (Bravo 500): 2004 two applications, 2005-2007 one application, rate 3l/ha
Azoxystrobin (Amistar): 2004 three applications, 2006 two applications, 2007 four applications, rate 1 l/ha

Supplementary Table S5. Crop protection protocols and fertilization regimes used in lettuce crops.

Fertilization**regimes**

Organic (OF) Composted cattle farmyard manure equivalent to 170 kg N/ha

Conventional (CF) Ammonium-nitrate (Nitram) equivalent to 150 kg N/ha and Superphosphate and KCl as a compound fertilizer (0:20:30) equivalent to 100 kg P₂O₅/ha and 150 kg K₂O/ha

Crop protection**protocols****Organic (OP)**

Weed control 2 × hoeing with tractor and front-end hoe, 3 × manual hoeing

Pest control None

Disease control None

Conventional (CP)

Weed control Propachlor at a rate of 9 l/ha once per year

Pest control none

Disease control Azoxystrobin (Amistar) at a rate of 1 l/ha twice per year

Supplementary Table S6. Crop protection protocols and fertilization regimes used in onion crops.

Fertilization**regimes**

Organic (OF) Composted cattle farmyard manure equivalent to 170 kg N/ha

Conventional (CF) Ammonium-nitrate (Nitram) equivalent to 150 kg N/ha and Superphosphate and KCl as a compound fertilizer (0:20:30) equivalent to 100 kg P₂O₅/ha and 150 kg K₂O/ha

Crop protection**protocols****Organic (OP)**

Weed control 2 × hoeing with tractor and front-end hoe, 4 × manual hoeing

Pest control None

Disease control None

Conventional (CP)

Weed control Propachlor at a rate of 9 l/ha once per year

Pest control none

Disease control Rovral Flo 2 l/ha three times in all three growing seasons
Azoxystrobin (Amistar) at a rate of 1 l/ha four times in 2005, three times in 2006 and four times in 2007

Supplementary Table S7. Proportion of variation explained, *F*-values and *p*-values of explanatory variables of a redundancy analysis (RDA) with radiation (RAD), air temperature (TEMP), precipitation (PRE) as environmental explanatory variables, and fertilizer types (organic [OF] vs mineral NPK [CF]), and crop protection protocols (organic [OP] vs conventional [CP]) as agronomic explanatory variables, and crop yield and selected nutritional quality parameters¹ as response variables.

	Environmental explanatory variables			Agronomic explanatory variables			
				Fertilization		Crop Protection	
Crop species							
RDA results	RAD	TEMP	PRE	CF	OF	CP	OP
Potato²							
variation explained (%)	8.5	NC	22.4	8.0	8.0	1.2	1.2
<i>F</i> -values	7.2	NC	24.6	9.8	9.8	1.4	1.4
<i>p</i> -values	0.004	NC	0.002	0.002	0.002	0.196	0.196
Cabbage²							
variation explained (%)	26.9	25.4	NC	3.6	3.6	1.0	1.0
<i>F</i> -values	42.8	26.2	NC	6.2	6.2	1.7	1.7
<i>p</i> -values	0.002	0.002	NC	0.002	0.002	0.162	0.162
Lettuce²							
variation explained (%)		24.4	46.7	1.4	1.4	0.3	0.3
<i>F</i> -values	NC	62.4	65.7	3.7	3.7	0.7	0.7
<i>p</i> -values	NC	0.002	0.002	0.010	0.010	0.638	0.638
Onion²							
variation explained (%)	NC	15.5	56.6	1.5	1.5		
<i>F</i> -values	NC	39.7	95.0	3.9	3.9	0.7	0.7
<i>p</i> -values	NC	0.002	0.002	0.010	0.010	0.540	0.540

RAD, radiation; TEMP, air temperature; PRE, precipitation; OF, cattle manure used as fertilizer; CF, mineral NPK used as fertilizer; OP, crop protection based on mechanical weed control and hand weeding in all crops, use of insect proof crop covers in cabbage crops, and application of Cu-fungicides in potato crops; CP, crop protection based on application of synthetic chemical herbicides and fungicides in all crops, and insecticides/nematicides in potato and cabbage crops; NC, not computed due to insufficient additional variation; ¹, response variables used in the RDA were the same as those included in the RDAs that produced the biplots shown in Figure 1; ², explanatory variables included in the RDA accounted for 40, 57, 73 and 74% of total variation for potato, cabbage, lettuce and onion respectively.

Supplementary Table S8. Proportion of variation explained, *F*-values and *p*-values of explanatory variables of a redundancy analysis (RDA) with radiation (RAD), temperature (TEMP), precipitation (PRE) as environmental explanatory variables, and nitrogen (N), phosphorus (P) and potassium (K) availability/supply (estimated from N, P, K concentrations in harvested potato tubers, cabbages and lettuce heads and onion bulb) and crop protection protocols (organic [OP] vs conventional [CP]) as agronomic explanatory variables, and crop yield and selected nutritional quality parameters¹ as response variables.

Agronomic explanatory variables, and crop yield and selected nutritional quality parameters as response variables.											
Crop species (fertilizer type)		RDA results	Environmental explanatory variables			Agronomic explanatory variables				variation explained ² (%)	
			RAD	TEMP	PRE	Nutrient availability			Crop Protection		
						N	P	K	CP	OP	
Potato (NPK)	variation explained (%)	16.4	8.5	NC	1.0	0.8	16.1	4.5	4.5	47.3	
	F-values	9.7	4.0	NC	0.6	0.5	7.1	2.8	2.8		
	p-values	0.002	0.004	NC	0.634	0.714	0.002	NC	0.026		
Potato (FYM)	variation explained (%)	11.6	13.1	NC	2.6	1.6	22.2	4.3	4.3	55.5	
	F-values	7.7	7.3	NC	1.8	1.1	10.6	3.0	3.0		
	p-values	0.002	0.004	NC	0.090	0.302	0.002	NC	0.032		
Cabbage (NPK)	variation explained (%)	NC	26.1	29.1	2.1	0.4	1.8	1.2	1.2	60.7	
	F-values	NC	13.1	23.4	1.7	0.3	1.5	1.0	1.0		
	p-values	NC	0.002	0.002	0.120	0.890	0.192	0.392	NC		
Cabbage (FYM)	variation explained (%)	7.7	NC	19.0	1.3	2.2	28.1	0.6	NC	58.9	
	F-values	5.9	NC	12.9	1.1	1.7	14.5	0.5	NC		
	p-values	0.002	NC	0.002	0.330	0.144	0.002	0.764	NC		
Lettuce (NPK)	variation explained (%)	NC	27.2	45.1	1.1	0.8	0.8	1.0	1.0	76.1	
	F-values	NC	34.4	29.6	1.5	1.0	1.1	1.4	1.4		
	p-values	NC	0.002	0.002	0.210	0.372	0.310	NC	0.240		
Lettuce (FYM)	variation explained (%)	25.4	NC	45.7	2.6	2.2	0.2	0.6	0.6	76.6	
	F-values	30.7	NC	30.3	3.4	2.9	0.3	0.8	0.8		
	p-values	0.002	NC	0.002	0.014	0.028	0.962	0.522	NC		
Onion (NPK)	variation explained (%)	NC	17.1	54.5	1.2	1.1	2.7	NC	0.6	77.3	
	F-values	NC	21.1	43.1	1.7	1.5	3.6	NC	0.8		
	p-values	NC	0.002	0.002	0.144	0.186	0.022	NC	0.476		
Onion (FYM)	variation explained (%)	16.7	NC	49.6	1.6	1.0	1.8	NC	0.3	70.9	
	F-values	17.8	NC	36.4	1.8	1.1	1.9	NC	0.3		
	p-values	0.002	NC	0.002	0.146	0.332	0.102	NC	0.940		

RAD, radiation; TEMP, temperature; PRE, precipitation; NPK, mineral NPK fertilizer; FYM, composted farmyard manure fertilizer; N, estimated nitrogen availability/supply; P, estimated P-availability/supply; K, estimated K-availability/supply; OP, crop protection based on mechanical weed control and hand weeding in all crops, use of insect proof crop covers in cabbage crops, and application of Cu-fungicides in potato crops; CP, crop protection based on application of synthetic chemical herbicides and fungicides in all crops, and insecticides/nematicides in potato and cabbage crops; NC, not computed due to insufficient additional variation; ¹, response variables used in the RDA were the same as those included in the RDAs that produced the biplots shown in Figure 1; ², proportion of total variation accounted for by the explanatory variables included in the RDA.

Supplementary field experiment

Introduction

Results from the first onion crops grown in the NFSC trials in 2003 identified (i) no significant effect of fertilization and crop protection regimes on onion yields and (ii) very low levels of pests and diseases damage. A supplementary pilot field trial was therefore carried out in 2004, to investigate whether changing the type of organic fertilizer used and/or increasing organic fertilizer input levels could increase onion yields without increasing pest and disease incidence/damage.

Material and methods

Experimental design

The experimental plots were located at the Stockbridge Technology Centre (STC, Cawood, Yorkshire, UK), which is in a region with fertile soils and intensive field vegetable, including onion, production. Onion crops were grown after 1-year red-clover fertility building ley.

Three organic fertilizer types were used (i) chicken manure pellets (commercial product, Greenvale, Yorkshire, UK, 4.4 % N), (ii) fresh farmyard manure (0.44 % N, from an organic farm near the trial site) and (iii) composted farmyard manure (from Nafferton Farm, Newcastle, UK 1.18 % N). Fertilizers were applied at three input levels (85, 170 or 250 kg N/ha) either individually or as combinations of two different fertilizer types. When combinations of fertilizer types were used, half the total amount of N was supplied by each of the two fertilizer types.

A completely randomized block design with 4 replicates was used (Supplementary Figure 2) and in addition to the fertilized plots each block also included 2 non-fertilized control plots. Individual plots were 1.8 m wide (1 bed with 4 rows) and 8 m long.

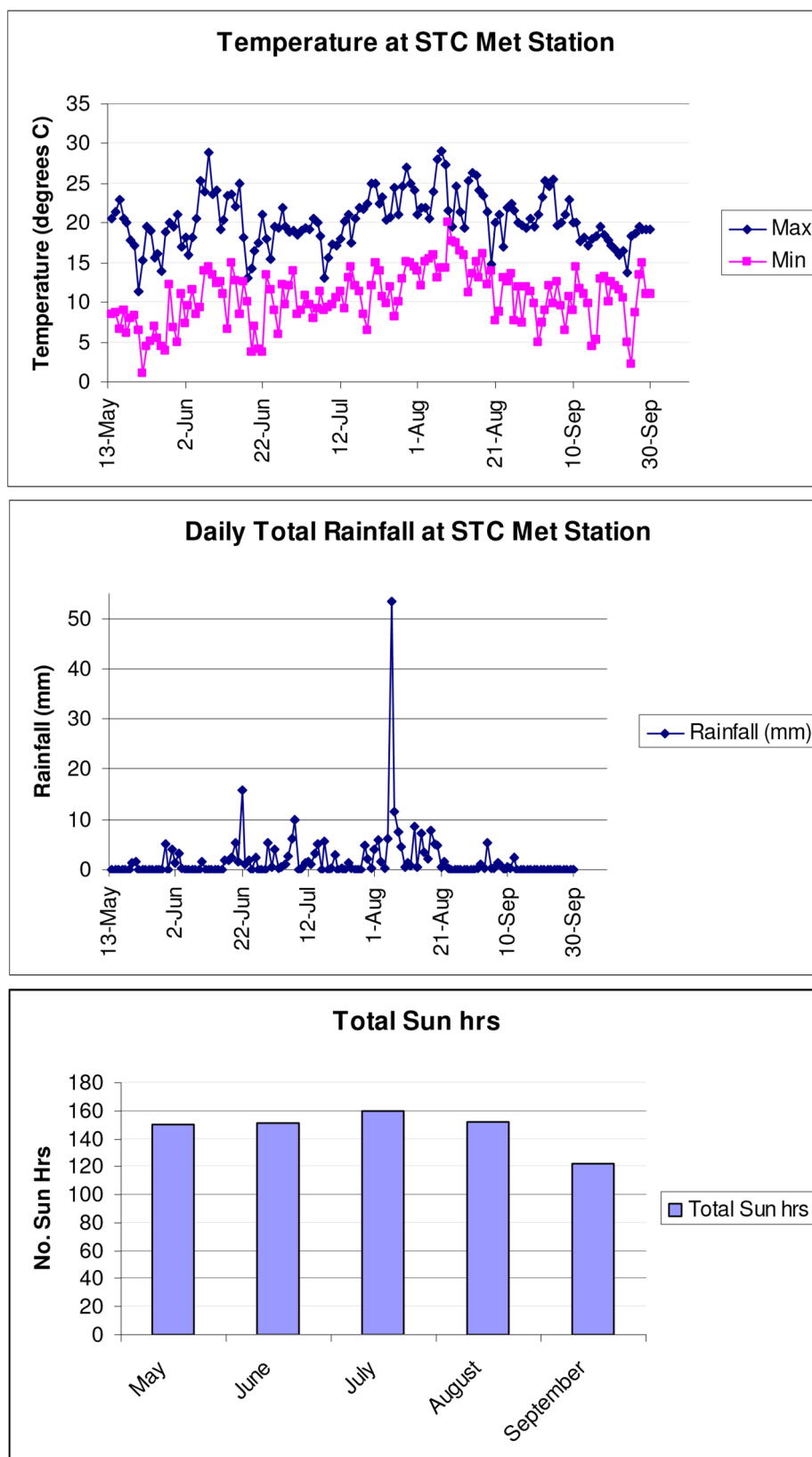
Block 3				Block 4			
D1	B3	C3	A1	G	F1	F2	D2
G	E1	E2	F2	A1	C1	E3	B3
D2	C1	G	A2	F3	B1	A2	E1
D3	B1	A3	F1	E2	C2	G	C3
E3	F3	C2	B2	D3	A3	D1	B2
E2	C1	A2	C3	A1	G	D1	G
D3	G	C2	F3	E1	C1	D2	E2
F1	B3	B1	G	B2	F2	B1	C3
B2	E3	A1	F2	D3	C2	E3	F1
D1	A3	D2	E1	F3	A2	B3	A3
Block 1				Block 2			

Supplementary Figure S2. Layout of the supplementary onion experiment.

Fertilizer input types used: A, chicken manure pellets (CMP); B, fresh farmyard manure (fFYM); C, composted farmyard manure (cFYM); D, CMP + fFYM; E, CMP + CFYM; G, no fertilizer input control.

Fertilizer input level: 1, 85 kg N/ha; 2, 170 kg N/ha; 3, 250 kg N/ha.

Fertilizers were manually spread evenly over the soil surface in each plot using a rake and then rotovated into the surface layer of the soil at the beginning of May. Onion transplants, c.v Spirit were then planted on the 14th of May and subsequently weeded by hand every 2-4 weeks. Plots were irrigated with 2.5 and 5 cm of water in June and July respectively. Climatic conditions (daily rainfall, daily max/min air temperature and sunshine hours/day) were monitored via the STC weather station throughout the duration of the trial (Supplementary Figure 3).



Supplementary Figure S3. Maximum and minimum daily temperatures (°C), daily rainfall (mm) and total monthly sunshine hours (Sun hrs) during the 2004 onion growing season at the Stockbridge Technology Centre (STC) site

During the initial 3 weeks after transplanting, the transplants became infested with bean seed fly (*Delia platura*) resulting in plant losses (see results sections below). When infestation became apparent all plots were treated with a garlic extract-based spray (approved under Soil Association organic farming standards standards), although this treatment was only partially effective at preventing further proliferation of the pest infestation.

Crop assessments

All plots were assessed weekly for visual symptoms of pests and disease infestations. Only bean seed fly (*Delia platura*) infestation and onion neck rot (caused by *Botrytis allii*) caused significant crop losses. They were therefore assessed in detail in each plot and recorded as the (i) % of plants lost due to bean seed fly infestation and (ii) the % of plants with neck rot symptoms (Supplementary Tables 9 and 10).

Total yields were assessed by harvesting, trimming and weighing all onion bulbs from the middle three meters. Marketable yields were determined by removing small and damaged bulbs, and bulbs showing visible symptoms of pest or disease infestation/damage using a commercial grading protocol (Supplementary Tables 9 and 10).

Nitrogen concentrations in onion bulbs were determined at Newcastle University by combustion using a LECO C&N analyzer (LECO corporation, St. Joseph, USA) according to the application notes provided by the instrument's producer (Form No. 203-821-273) (Supplementary Tables 9 and 10).

Statistical analyses

The effects of fertilizer type and input level on measured parameters were assessed using ANOVA derived from linear mixed-effects model using the 'nlme' package [38] in R [39] (Supplementary Table 9). To compare results obtained with fertilizer inputs with those recorded in unfertilised control plots we carried out separate 1-factor ANOVAs for each fertilizer type with fertiliser input level as the factor (Supplementary Table 10). Differences between means for fertilizer types and input levels were tested using Tukey contrasts with the general linear hypothesis testing (glht) function of the 'multcomp' package in R [41].

Supplementary Table S9. Effect of fertilizer input types and input level on total and marketable bulb yields, bulb N-content, bean seed fly damage and onion neck rot incidence in onion. Values shown are main effect means and are expressed on a fresh weight basis

	Total yield (t/ha)	Marketable Yield (t/ha)	Bulb N-content (%)	Bean seed fly (% lost plants)	Neck rot (% plants with symptoms)
Non fertilised¹	23.2	21.6	1.33	10	10
Fertilized (mean of all fertilizer treatments)	19.8	18.7	1.38	31	13
Input type²					
Chicken Pellets (CP)	12.4 b	11.9 c	1.35	50 b	21
Cattle Manure (fFYM)	23.6 a	22.0 ab	1.40	15 a	6
Compost (cFYM)	26.0 a	24.7 ab	1.34	16 a	8
CP + fFYM	15.9 b	15.5 bc	1.38	48 b	22
CP + cFYM	13.9 b	13.2 c	1.41	48 b	16
fFYM + cFYM	27.4 a	25.9 a	1.38	14 a	8
Input level²					
85 kg N ha ⁻¹	22.8 a	21.7 a	1.31 a	22.3 a	12

170 kg N ha ⁻¹	19.6 ab	18.6 ab	1.38 ab	32.1 ab	13
250 kg N ha ⁻¹	16.8 b	15.8 b	1.45 b	40.6 b	15

ANOVA (*p*-values)

Main Effects

Input type (IT)	<0.001	<0.001	NS	<0.001	<0.032
Input level (IL)	0.003	0.003	0.008	<0.001	NS
<u>Interaction (IT × IL)</u>	NS	NS	NS	0.035	NS

¹ data from non-fertilized control plots were not included in 2-way ANOVA; means with the same letter are not significantly different according to Tukey's Honest Significant Difference Test ($p < 0.05$).

Supplementary Table S10. Effect of fertilizer input level on total and marketable bulb yields, bean seed fly damage and onion neck rot incidence in onion crops fertilized with different organic fertilizer types. Values shown are main effect means and are expressed on a fresh weight basis

Fertilizer type		Total yield (t/ha)	Marketable Yield (t/ha)	Bean seed fly (% lost plants)	Neck rot (% plants with symptoms)
N-input level (kg/ha)					
Chicken manure pellets (CMP)					
	0	23.3 a	21.5	10 a	9.8
	85	15.7 ab	15.2	44 b	25.1
	170	11.3 ab	10.5	59 b	17.8
	250	10.2 b	9.8	48 b	20.8
	1-factor ANOVA (<i>p</i>-value)	0.008	0.013	<0.001	NS
Fresh farm yard manure (fFYM)					
	0	23.2	21.6	10	9.8
	85	23.5	21.7	6	5.9
	170	25.0	23.4	16	8.4
	250	22.3	20.9	23	3.5
	1-factor ANOVA (<i>p</i>-value)	NS	NS	NS	NS
Composted farm yard manure (cFYM)					
	0	23.2	21.6	10	9.8
	85	28.6	27.2	13	7.3
	170	21.9	20.6	16	10.5
	250	27.9	26.8	19	7.1
	1-factor ANOVA (<i>p</i>-value)	NS	NS	NS	NS
CMP + fFYM					
	0	23.2	21.6	10 a	9.8
	85	20.3	19.9	28 ab	22.3
	170	15.8	15.2	48 bc	17.9
	250	11.6	11.3	69 c	24.5
	1-factor ANOVA (<i>p</i>-value)	NS	NS	<0.001	NS
CMP+cFYM					
	0	23.2 a	21.6 a	10 a	9.8
	85	18.3 ab	17.2 ab	28 ab	5.4
	170	14.3 ab	13.8 ab	44 bc	16.8
	250	9.0 b	8.6 b	71 c	26.4
	1-factor ANOVA (<i>p</i>-value)	0.016	0.024	<0.001	NS
fFYM + cFYM					
	0	23.2	21.6	10	9.8
	85	30.5	29.1	16	8.2
	170	29.4	28.4	10	4.8
	250	22.4	20.3	15	9.8
	1-factor ANOVA (<i>p</i>-value)	NS	NS	NS	NS

means with the same letter are not significantly different according to Tukey's Honest Significant Difference Test ($p < 0.05$).