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Biostimulants and nitrogen management: an assessment of famer-led trials (Angus Smart Arable Farm 2021)

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CONTENTS

1. Abstract

This project supported the Angus Smart Arable Farm Group. Specifically, it aimed to enable them to meet trial objectives and answer questions set by the group's farmers. Activities within this project were designed to complement the farmer-led investigations to get the maximum benefit from the trials, data and analyses.

The winter wheat and spring barley trials were located at two farms in Angus, Scotland. In 2021, the trials aimed to evaluate ways to reduce nitrogen (N) fertiliser rate, while maintaining yields. The trials included Nurture N, which is marketed as a liquid fertiliser amendment.

Prior to the commencement of this project, the trials were established by the farmer group. This project commenced with a kick-off meeting, involving the researchers and participating farmers. At this meeting, the following was discussed: the farmers' hypotheses and questions, the trials, the potential assessments, and the value added to the overall understanding. Because treatments had been applied before this project commenced, it was too late co-design the trial layouts. However, it provided an opportunity to discuss robust trial designs with the group. During the trials, researchers conducted several site visits and facilitated crop assessments. Grower participants were provided with data at update meetings.

The winter wheat trials identified significant yield reductions in response to reducing nitrogen rates (by 60kg N/ha and 40 kg N/ha) at the two participating farms. In the Strathmore trial, the application of Nurture N and additional nutrient and biostimulant products had no effect on yield in the reduced nitrogen situation. Physiologically, the reduced nitrogen rate resulted in a significantly lower number of ears/m^2 and grains/m^2 in both trials. Notably, at Strathmore, the reduction in nitrogen rate also resulted in a lower thousand grain weight (TGW), whereas there was no effect of the reduced nitrogen treatment at Grange of Conon.

In contrast to the winter wheat trials, there was minimal effect of the reduced nitrogen treatment with Nurture N applied to spring barley at Grange of Conon. The results indicated that the current farm standard application rate may be in excess, offering scope to reduce rates. In the Strathmore experiment, the results showed a large yield increase (more than 1 t/ha) in response to the Nurture + treatment (note: this did not include a reduction in nitrogen rate). Because there was no replication, this result should be treated with caution.

Any further trials to understand how to reduce nitrogen rates while maintaining yields should build on the lessons from these initial trials. Future trials should include a focus on robust trial designs (with replication), the optimum application timings, rates of products being tested, as well as complimentary measurements to help explain yield effects.

2. Introduction

The aim of this project was to provide support to the Angus Smart Arable Farm Group to enable them to meet the trial objectives and questions that were framed at the start of the project. Activities within this project were designed to complement farmer-led investigations and help the farmers to get the maximum benefit from the trials, data and analyses.

The Smart Farm programme provides an opportunity for farmers to innovate which is backed up by independent research. It provides a platform for growers to discuss and share their experiences and encourage the incorporation of evidence-based innovations into their businesses. The Smart Farm approach aims to bridge the gap between research and practical farming and provide a programme of farmer led trials/demonstrations, which are relevant to the current situation facing UK farming. This approach is designed to provide robust information to farmers to help inform decisions for changes to practice on their own farms.

With a global focus on sustainability and the impact of food production on the environment, understanding the impact of inputs applied to crops on both production and the environment is paramount and of significant interest to farmers in the UK. Inorganic nitrogen (N) fertilisers can contribute to significant yield increases, whilst also having the potential to cause serious environmental implications. N fertiliser is responsible for a significant proportion of green house gas (GHG) emissions that are associated with crop production (Mahmuti et al., 2009; Berry et al., 2010), as well as contributing to water and air pollution (Davies and Sylvester-Bradley, 1995; Misselbrook et al., 2000).

The focus of the trials in this programme was to evaluate routes to reducing nitrogen fertiliser rates in winter wheat and spring barley whilst maintaining yields. Across two farms located in Angus, Scotland, trials were performed to determine if the Aiva Fertiliser product Nurture N could contribute positively to maintaining yields in a reduced N situation. The manufacturer describes Nurture N as a liquid fertiliser amendment that is an enhanced carbohydrate material, high in organic acids, proteins and Cation Exchange Capacity (CEC). The product is largely comprised of fulvic and humic acids, as well as quantities of chlorides, potassium, sodium, sulphur, calcium and magnesium as well as traces of copper, iron, manganese, phosphorus and zinc. At each farm, a winter wheat and a spring barley trial were performed to understand the impact of reducing N rate and the use of Nurture N (Table 1). In one of the winter wheat trials, a number of additional nutrient and biostimulant products were also applied. This also included the Aiva product Nurture 60, which is described as a containing fulvic and humic acids, macro and micro nutrients and has significant plant bio-stimulant properties which provide an effective and available source of carbon energy and carbohydrates to feed and stimulate the growth of beneficial microorganisms.

There is considerable interest in the use of a wide range of biostimulant products that are currently available across a broad range of crop types in the UK. The term 'biostimulant' covers everything that can be added to the plant or soil to stimulate natural processes to benefit the plant, beyond fertilisation or pesticidal action alone. A review by Storer et al (2016) highlighted that there are many gaps in the understanding of the benefits of biostimulants for cereals and oilseed rape crops in the UK, and field data for these crops is lacking for many biostimulant product types.

Humic substances (HS) are the product of natural decomposition of plant and microbial remains and comprise up to 80% of soil organic matter. HS are complex mixtures of polydispersed materials, which can be split into three main categories: humic acids (HA), fulvic acids (FA) or humin. The use of soluble HS as plant growth promoters is not novel, however they are often applied with other fertiliser products and/or in situations of nutrient deficiency, which makes it difficult to discern any biostimulant effects. The biostimulant effects of HS are characterised by both structural and physiological changes in roots and shoots related to nutrient uptake, assimilation and distribution (nutrient use efficiency traits) (Canellas et al., 2015). The enhancement of N uptake/assimilation and N metabolism in plates treated with HS has been documented in barley (Piccolo et al., 1992, Albuzio et al., 1986). Humic acid might also benefit plant growth by chelating unavailable nutrients and buffering pH (Mackowiak et al., 2001). However, high doses of HS can have negative effects on plant growth (Asli and Neumann 2010; Ayuso et al.,1996). Tahir et al., (2011) found that application of lignite-derived humic acid at a high dose had a negative effect on the growth and nutrient uptake of wheat, as well as nutrient accumulation in the soil in comparison to lower doses.

A kick off meeting was organised where the participating farmers discussed their hypotheses and questions, the trials that they had set up, potential assessments and the value that they added to overall understanding. Data was provided back to the growers following a number of update meetings (attended through MS Teams, Table 2). The aim of these meetings, as well as regular contact between researchers and the farmers, was to ensure that data and information was reviewed on a regular basis, with the view to using the data to inform decisions, rather than contributing to just a review at the end of the season. Unfortunately the commencement of the project was too late for the co-design of the trial layouts as treatments had already been applied, however useful information was discussed regarding robust trial designs.

Table 1: Sites and treatments

Site	**Farm Standard N (FS)**	**Treatment 1**	**Treatment 2**
WW1 (Strathmore)	190kg N/ha	**N-60** 1.5l/ha + 1l/ha Nurture N minus 60 kg N/ha plus 5l/ha Nurture 60, 10l/ha Special 1230, 1l/ha Syngergy, 1l/ha Mobo	**FS-60** FS-60 kg N/ha
WW2 (Grange of Conon)	200kg N/ha	**N-40** 1.5l/ha Nurture N minus 40kg N/ha	
SB1 (Strathmore)	126kg N/ha	**Nurture +** 1.5l/ha Nurture N plus 5l/ha Nurture 60, 10l/ha Special 1230, 1l/ha Synergy, 1l/ha Mobo	
SB2 (Grange of Conon)	130 kg N/ha	**N-20** 1.5l/ha Nurture N minus 20kg N/ha	

Table 2 – record of meetings

Date	Aim of meeting	Results presented	Other topics discussed
27th May 2021	Kick off meeting		Trial questions and hypotheses. Reduced N.
22nd June 2021	Meeting 2	Results from first sampling	Efficacy of foliar treatments.
29th July 2021	Meeting 3	Results from second sampling	Value of Grain nutrient sampling.
9th November 2021	Meeting 4	Results from Harvest	Benefits of good experimental design. Nitrogen use efficiency.

3. Materials and methods

On-site sampling was performed by SAC Consulting, and where relevant, samples were sent to ADAS Gleadthorpe for further processing.

Table 3: Summary of assessments performed at each trial site.

Site	Visit 1 June	Visit 2 Mid grain fill	Visit 3 Pre Harvest	Harvest
W.Wheat	Shoot numbers Disease score Sample for tissue tests Satellite NDVI	Green area score Disease score Satellite NDVI	Quadrat samples ears/m^2, grains/ear, grains/m^2, TGW, DMHI	Yield (from combine yield map) Grain samples for grain nutrient analysis and Fatty Acid analysis
Spr.Bar	Shoot numbers Disease score Sample for tissue tests Satellite NDVI	Ears/m^2 Green area score Disease score Satellite NDVI		Yield (from weighbridge) Grain samples for grain nutrient analysis and Fatty Acid analysis

3.1. Visit 1 – June 2021

A representative sample of the newest fully expanded leaf was taken from each treatment in both the winter wheat and spring barley trials. Samples were sent to Lancrop Laboratories for nutrient analysis.

The number of shoots/m^2 was measured by counting the number of shoots in 2 x 0.5m rows at 10 positions in each treatment tramline.

3.2. Visit 2 – Mid grain fill July 2021

Ear numbers were counted from 2 rows x 0.5m using a clapperboard technique and repeated 10 times per treatment tramline. One ‘typical tiller was selected from each sample point and taken back to the laboratory. For each tiller the height of the stem to the base of the ear was measured as well as the ear length (not including awns). Leaves were removed by leaf layer and assessed for disease, leaf length and maximum width. A greenness scale was constructed by taking the greenest and yellowest leaves from the whole trial area and individual leaves were then scored on a scale of 1 to 10. Leaf area index was calculated from leaf dimensions and tiller numbers. Green area index was estimated from leaf area index and the proportion of green leaf remaining from the greenness scale.

3.3. Visit 3 – Pre-harvest August 2021

Whole crop samples were cut at ground level from 4 rows x 0.75m (0.45m2) and at 5 sample points per treatment tramline for the winter wheat trials only. Samples were transported to ADAS laboratories for further processing. Fertile and infertile shoots were counted, ears removed and both straw and ears were dried in an oven at 85^{0}C for 48hrs before weighing. Ears were threshed, grain weighed and TGW measured to enable calculation of grain numbers per ear. Ears/m^2 and grains/m^2 were calculated using combine yields.

3.4. Harvest

Where combine yield mapping data had been provided analysis was carried out using the ADAS Agronomics statistical method. Where yield maps were not available farmers took a series of whole header cuts of known length through each treatment and weighed on a weighbridge. Yields were corrected to 85% DM from combine grain samples. Grain samples from each strip were analysed through YEN Nutrition, whereby samples were sent to NRM for nutrient analysis. Additionally, grain samples from the winter wheat and spring barley trials at Grange of Conan were sent to Sciantec for fatty acid analysis to obtain further understanding about the nutritional value of the crops.

3.5. NDVI Imagery

NDVI imagery was obtained from Data Farming to monitor the impact of treatments across the winter wheat and spring barley trials.

3.6. Statistics

Collected data was analysed by either a paired t-test or ANOVA using Genstat 18th edition. Where the farmers had access to a yield mapping combine, data was analysed using the ADAS Agronomics methodology, to robustly understand the treatment effects on yield.

4. Results

4.1. Disease and Leaf area index

In the Strathmore winter wheat trial, it was very apparent that the % green area was reduced in response to the reduced N application (FS-60), but that this was then reduced further with the addition of Nurture N plus additional products (N-60) (Figures 1 and 2. Table 4). On the Flag leaf, green area was reduced from 98% to just 60% in the N-60 treatment, with greenness score also reduced in this treatment. Both LAI and GAI were significantly ($P<0.001$) affected by the treatment (Figure 1), with both the N-60 and the FS-60 treatments having significantly lower GAI than the FS treatment. Similarly, in the winter wheat trial at Grange of Conon, GAI and LAI were also significantly affected by the N-40 treatment (Figures 3 and 4, Table 4). There was little difference in the green area or greenness score for the Flag Leaf, whilst the N-40 treatment appeared to reduce the green area and greenness of leaf 2, and green area of leaf 3.

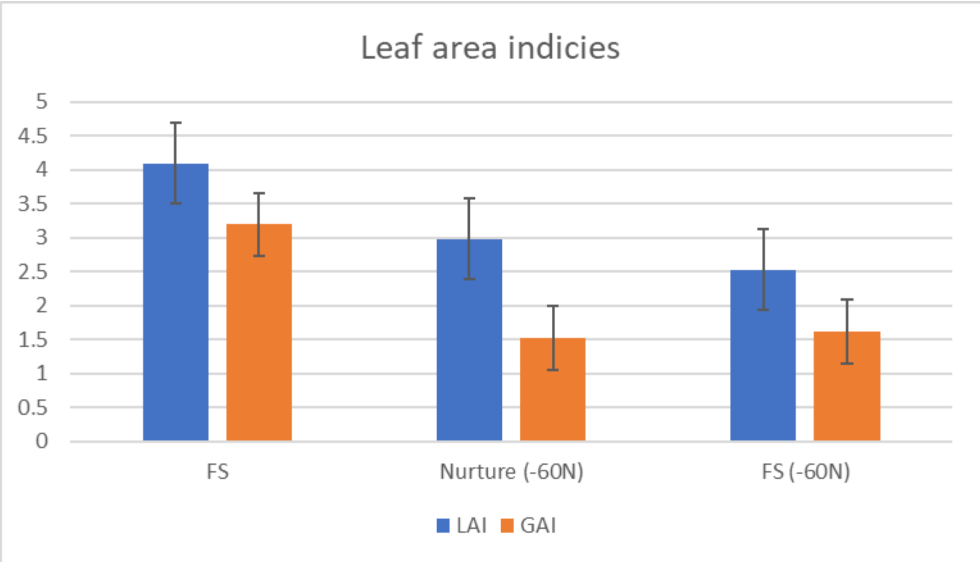

Figure1. LAI and GAI for winter wheat trial at Strathmore. Error bars indicate LSD.

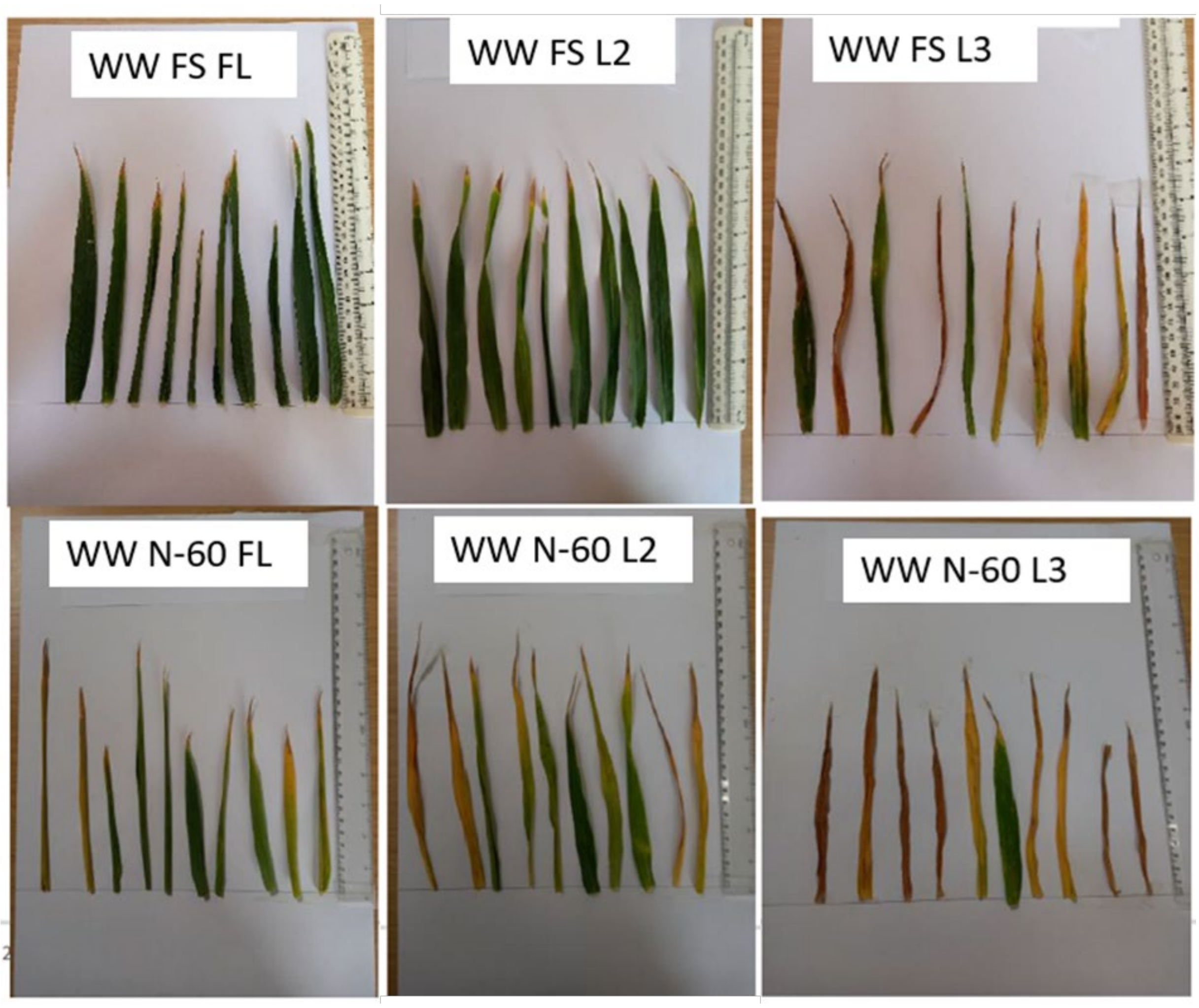

Figure 2. Photographs of the Flag Leaf (FL), Leaf Two (LT) and Leaf Three (LT) from the winter wheat trial at Strathmore for the FS and N-60 treatments.

Table 4. Disease and Leaf area index. FL: Flag leaf; L2: Leaf 2; L3: Leaf 3 for winter wheat and spring barley trials.

Site	Treatment	Green area (%)			Greenness (1-10)			Disease (%)		
		FL	L2	L3	FL	L2	L3	FL	L2	L3
Strathmore WW	FS	98	96	20	9.5	8.5	8	0	3	6
	FS-60	90	70	5	8.5	7.5	3	2	8	7
	N-60N	60	60	15	7.5	7.5	5	2	5	8
GOC WW	FS	85	45	5	8.5	7	2	4	6	10
	N-40N	88	25	2	8.5	6	2	4	7	15

Strathmore SB	FS	9	5	0	6	2	2	15	10	5
	N+	8	0	0	4	0	0	20	15	5
GOC SB	FS	92	85	15	9	7	2	2	1	6
	N-20N	75	75	5	7.5	7	1	5	4	8

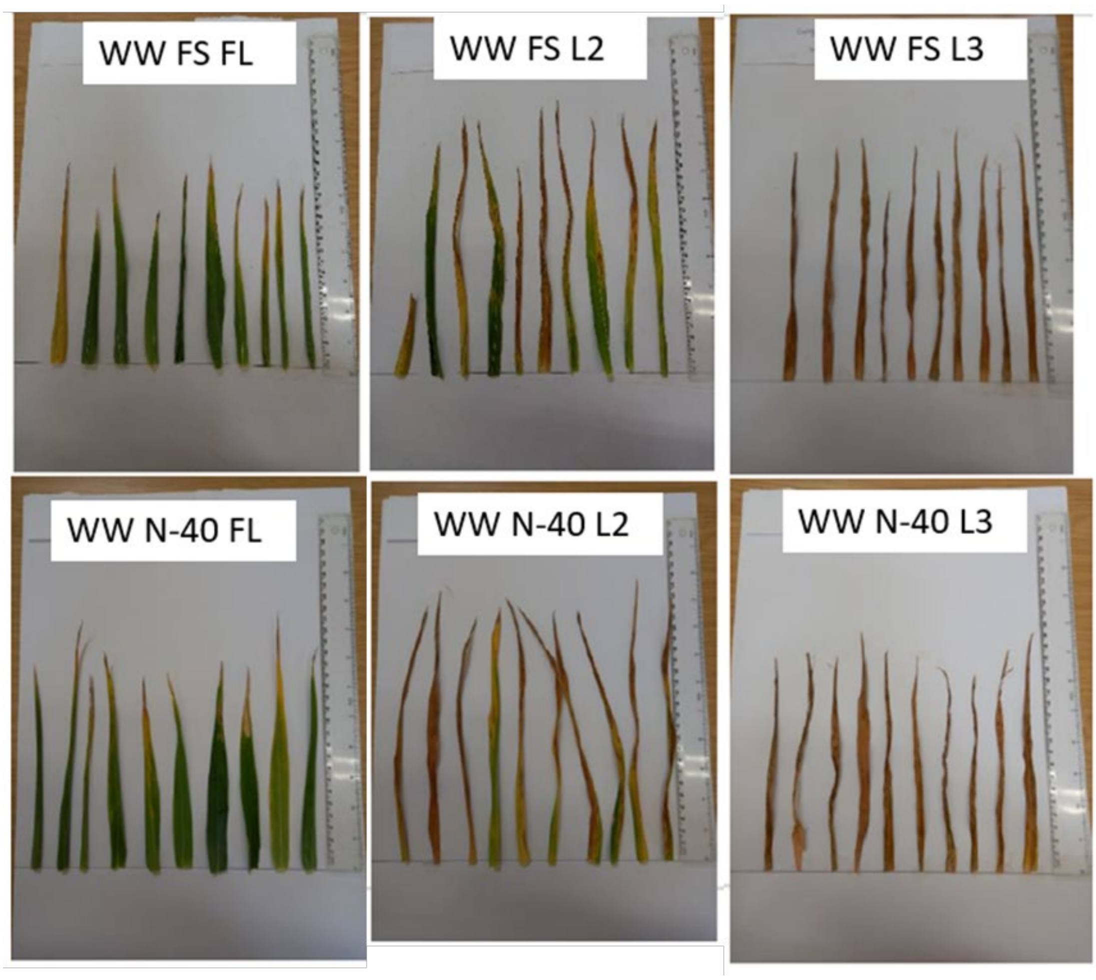

Figure 3. Photographs of the Flag Leaf (FL), Leaf Two (LT) and Leaf Three (LT) from the winter wheat trial at Grange of Conon for the FS and N-40 treatments.

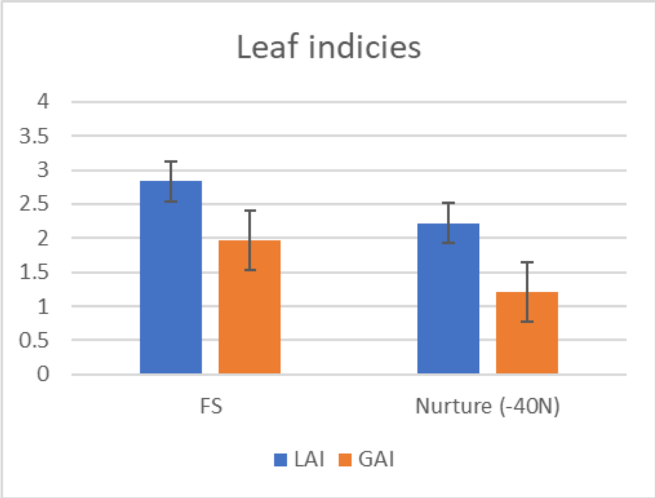

Figure 4. LAI and GAI for the winter wheat trial at Grange of Conon. Error bars indicate LSD.

In the spring barley trial at Strathmore, LAI was not significantly affected by the Nurture N + treatment (Figure 5). Whilst the GAI was extremely low, there was a significant reduction (P<0.001) in the GAI in the Nurture N + treatment in comparison to the FS treatment. In the Grange of Conon trial, both GAI and LAI were significantly reduced in response to the N-20 treatment (Figure 6).

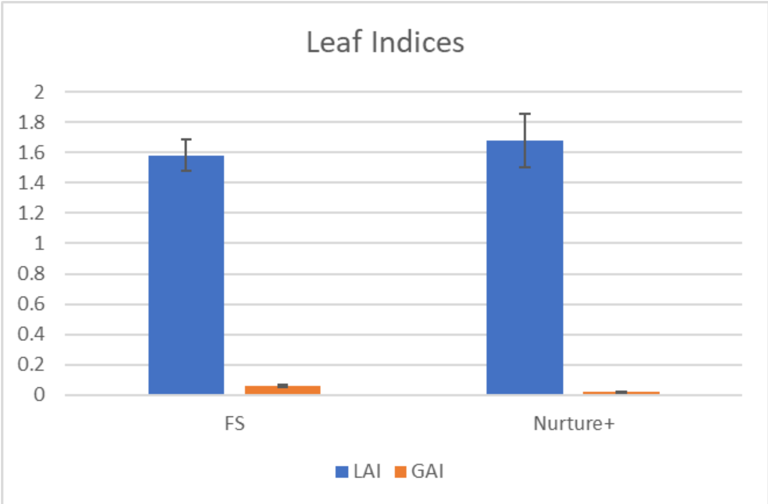

Figure 5. LAI and GAI in the spring barley at Strathmore. Error bars represent the LSD.

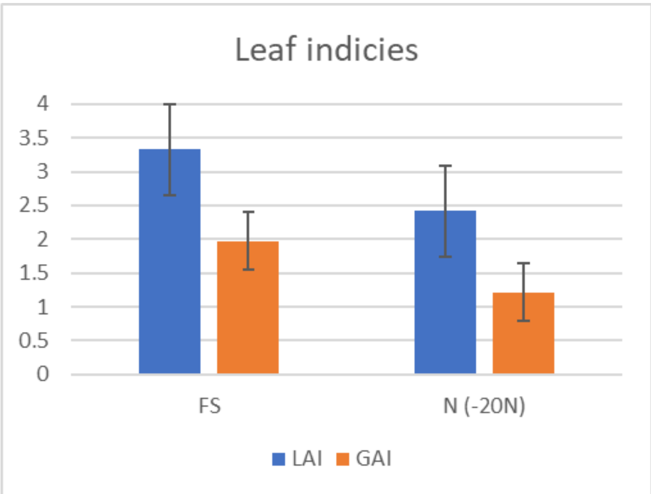

Figure 6. LAI and GAI in the spring barley at Grange of Conon. Error bars represent the LSD.

Plant height and ear length were measured in the tillers selected for assessment in both the winter wheat and spring barley trials. At the Strathmore site, stem height was significantly reduced in response to the N-60 treatment and the FS-60 treatment, whilst there was no significant effect on ear length (Figure 7). Similarly, at Grange of Conon, stem height was significantly reduced in response to the N-40 treatment, whilst there was no effect on ear length (Figure 7).

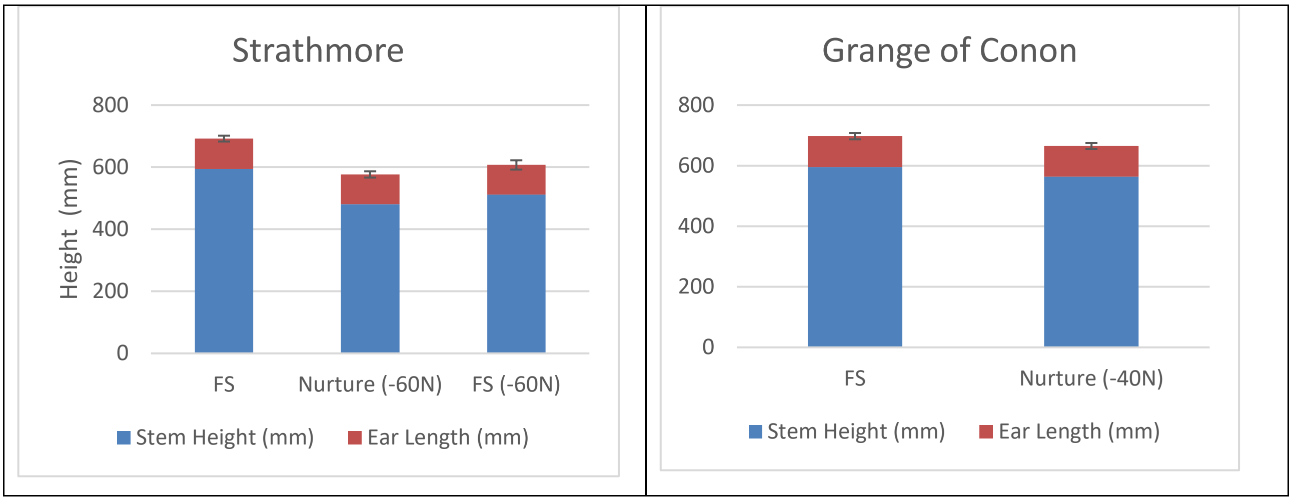

Figure 7. Plant height and Ear length (mm) measured in the winter wheat trials (Error bars represent SED.

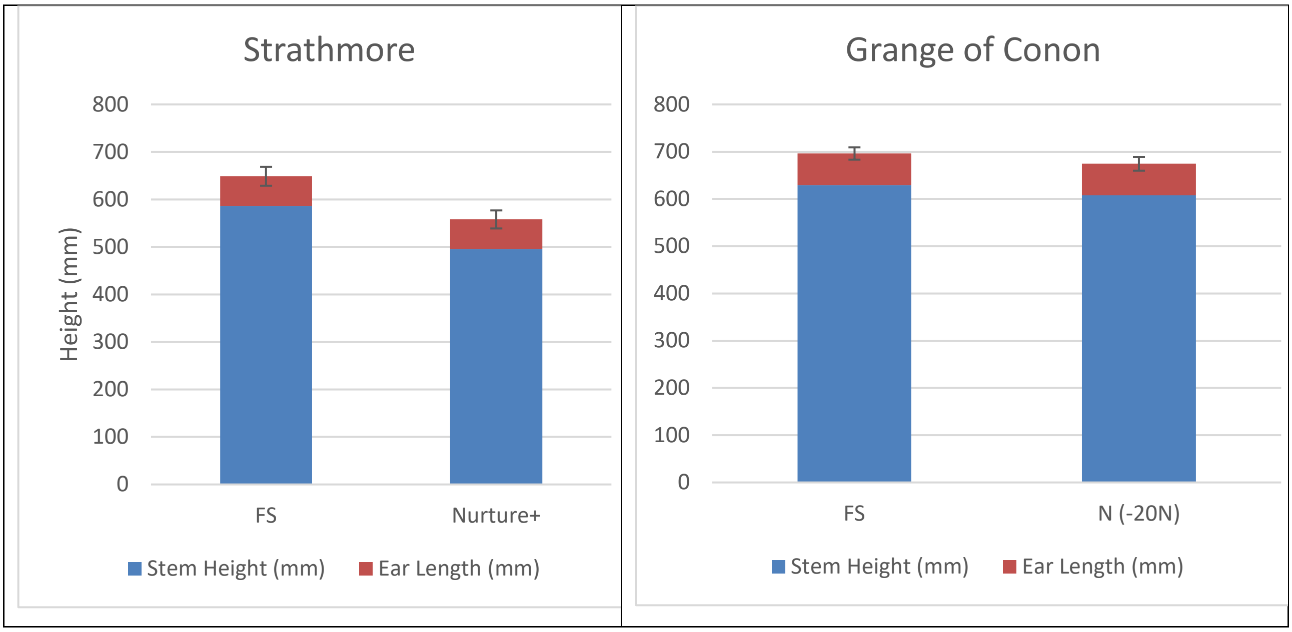

Figure 8. Plant height and Ear length (mm) measured in the Spring barley trials (Error bars represent the SED.

In the spring barley trial at Grange of Conon, there was no significant effect of the N-20 treatment on stem height or ear length, whereas at Strathmore, the N+ treatment resulted in a significantly shorter stem (Figure 8). There was no significant effect of the N+ treatment on ear length at Strathmore.

4.2. NDVI

Imagery clearly showed that both the FS-60 (N2) and the N-60 (N1) treatments resulted in reduced NDVI in both June and July in the winter wheat trial at Strathmore, and this was also visually noticeable in the field also (Figure 9). At Grange of Conon, it was also possible to distinguish the reduced N treatment tramline in the field from satellite imagery in June and July, however, this difference was much more subtle. In the spring barley trials, at Strathmore, the Nurture N+ treatment did not show obvious effects on NDVI, and this was also true in the spring barley trial at Grange of Conon.

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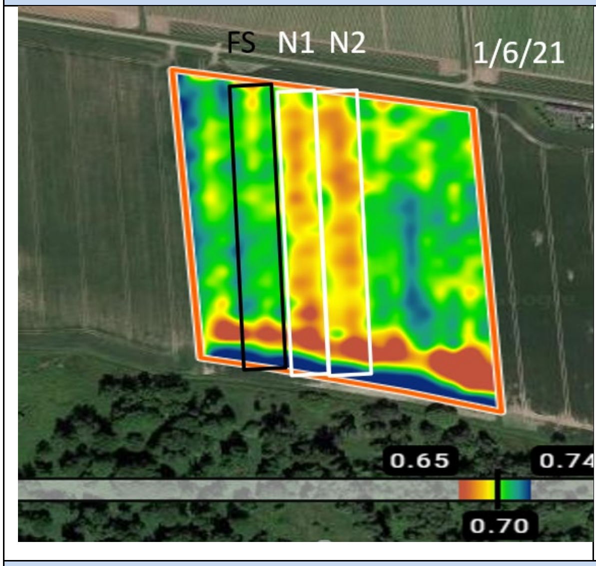
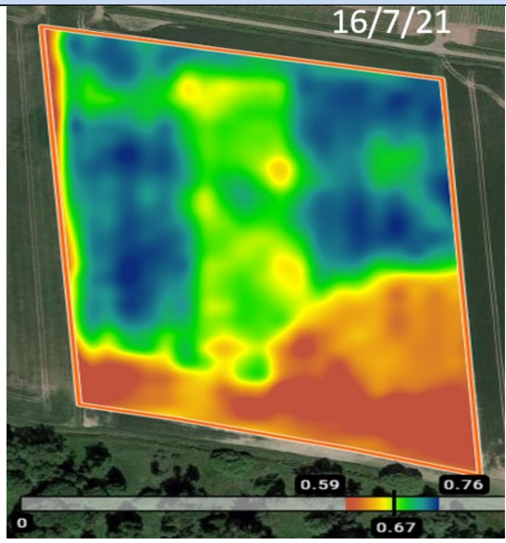
<tr><td>
</td><td>
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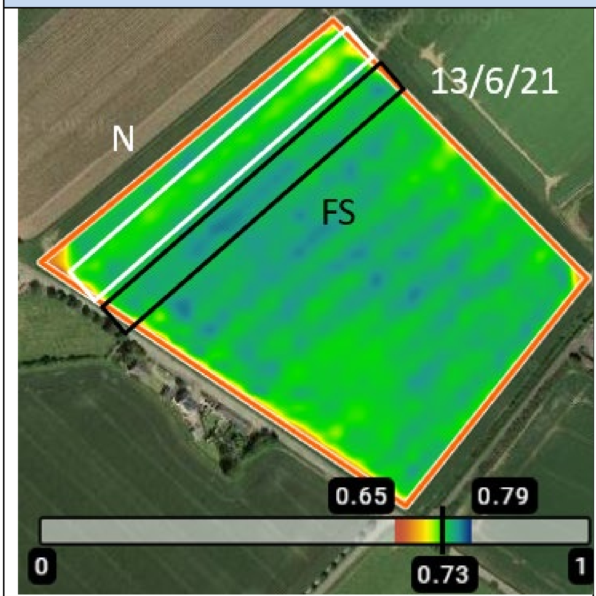
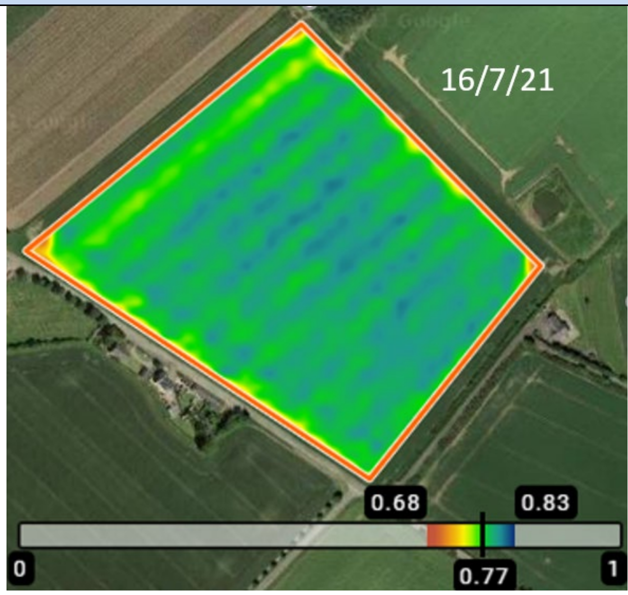
<tr><td colspan="2">NDVI – Grange of Conon winter wheat</td></tr>
<tr><td>
</td><td>
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<tr><td></td><td></td></tr>
<tr><td colspan="2">NDVI – Strathmore spring barley (red areas on 1/7/21 denote cloud cover)</td></tr>
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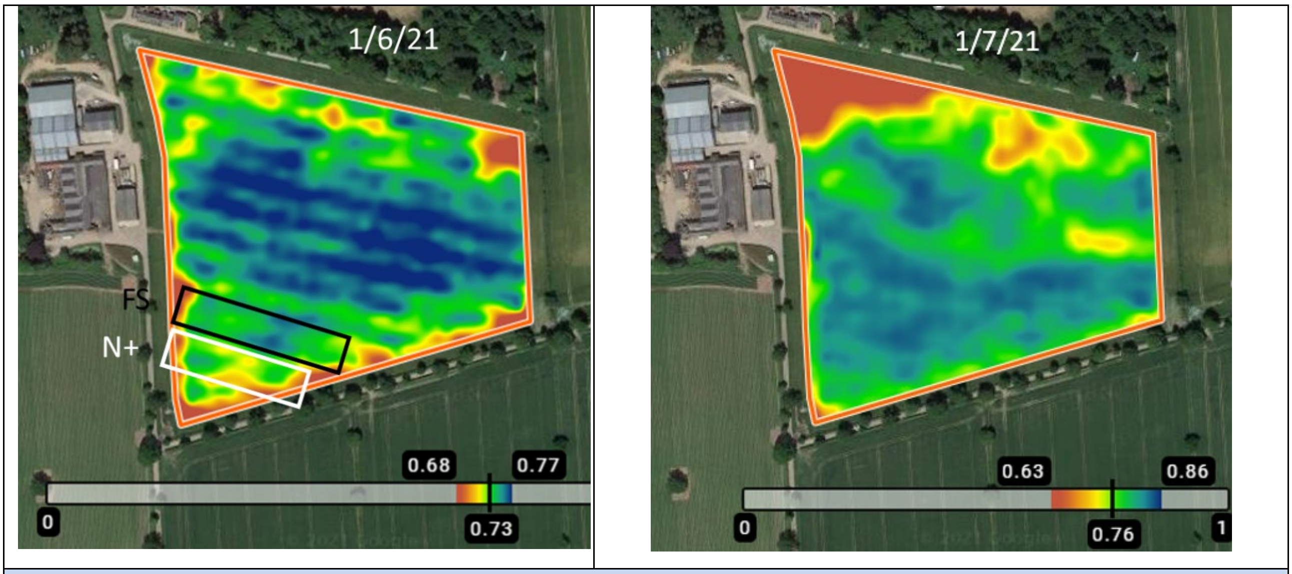

NDVI – Grange of Conon spring barley

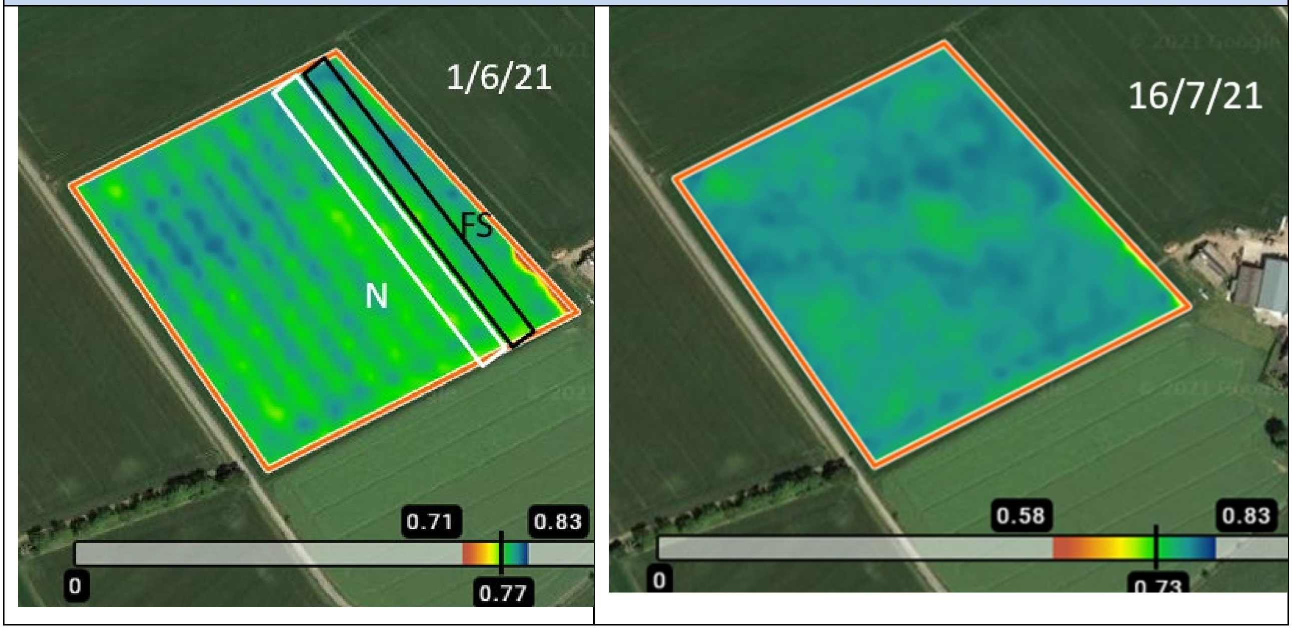

Figure 9 – NDVI imagery for the two winter wheat and two spring barley trials taken from www.datafarming.com.

4.3. Tissue testing

Leaf samples were sent to Lancrop laboratories for tissue analysis, and the results highlighted a range of nutrients that were either slightly low, low or very low, according to Lancrop's Guideline values and thresholds (Tables 5 and 6). At Strathmore, the concentration of Ca, Mg, Cu, Fe, Mn and Zn were all adequate in the winter wheat trial across all three treatments. Levels of Mo were low across all three treatments. The FS-60 treatment had a very similar concentration of N in comparison to the FS treatment, and this was slightly higher in the N-60 treatment. At Grange of Conon, the N-40 treatment still had an adequate concentration of N in the leaf, although this was lower than the FS treatment. Concentrations of N, P, Mg, S, Fe, Mn and Mo were all adequate. At this site, levels of B were very low across both treatments, with Cu and Zn on the low side, and K and Ca coming out as slightly low.

Table 5 Tissue analysis Winter wheat

		Strathmore				Grange of Conon	
	Guideline	FS	FS-60	N - 60		FS	N -40
N (%)	3	3.4	3.38	4.34		4.2	3.71
P (%)	0.3	0.28	0.26	0.3		0.39	0.39
K (%)	3.5	2.53	2.66	3.04		2.85	2.87
Ca (%)	0.4	0.46	0.39	0.49		0.37	0.31
Mg (%)	0.12	0.14	0.14	0.16		0.14	0.12
S (%)	0.25	0.32	0.33	0.42		0.38	0.33
B (ppm)	6	4.8	3.8	4.3		2.9	2.1
Cu (ppm)	7	5.9	4.4	5.4		4.7	4.7
Fe (ppm)	50	121	97	126		117	105
Mn (ppm)	35	46	39.2	65.3		49.3	46.5
Mo (ppm)	0.1	1.07	0.86	0.68		0.84	0.74
Zn (ppm)	25	14.6	17.9	13.4		17	15

Slightly low	Low	Very low

Tissue analysis results for the two spring barley trials also provided a useful insight into the nutritional status of the crop at the time of sampling. At Strathmore, the crop was low in P and Cu across both treatments. The Nurture N+ treatment generally showed very similar nutrient levels to the FS treatment, with the exception of Mo. Concentrations of K, Mg, B and Zn were also slightly low across both treatments. At Grange of Conon, it was notable that considerably fewer nutrients were below the guideline concentration. These included B which was low and Mg which was slightly low across both treatments. Cu was slightly low in the FS treatments, but in the N-20 treatment Cu levels were adequate.

Table 6 Tissue analysis Spring Barley

	Strathmore				Grange of Conon	
	Guideline	FS	Nurture +		FS	N-20
N (%)	2.8	3.5	3.59		2.8	4.23
P (%)	0.35	0.24	0.25		0.4	0.4
K (%)	3	2.56	2.69		3.54	3.26
Ca (%)	0.5	0.49	0.54		0.56	0.53
Mg (%)	0.15	0.12	0.12		0.12	0.12
S (%)	0.2	0.39	0.38		0.34	0.34
B (ppm)	6	4.6	4.8		3.9	4.1
Cu (ppm)	6	3.6	3.6		5.9	6.3
Fe (ppm)	50	110	107		91	90
Mn (ppm)	30	51	48.8		137	106.7
Mo (ppm)	0.1	0.36	0.62		0.44	0.42
Zn (ppm)	20	15.2	14.9		28.9	28.7

	Slightly low		Low		Very low

4.4. Yield

The yield data for the winter wheat trial at Strathmore was analysed using the ADAS Agronomics approach. First the data was cleaned to remove headlands, anomalous combine runs (header not full or spanning two treatment areas) and locally extreme data points, and to correct any offset created by changes in combine direction. Then a model of underlying variation was applied to the data to account for spatial variation across rows and along rows, and for the effect of the treatment. The statistical analysis returned treatment effects with standard errors, allowing calculation of 95% confidence limits.

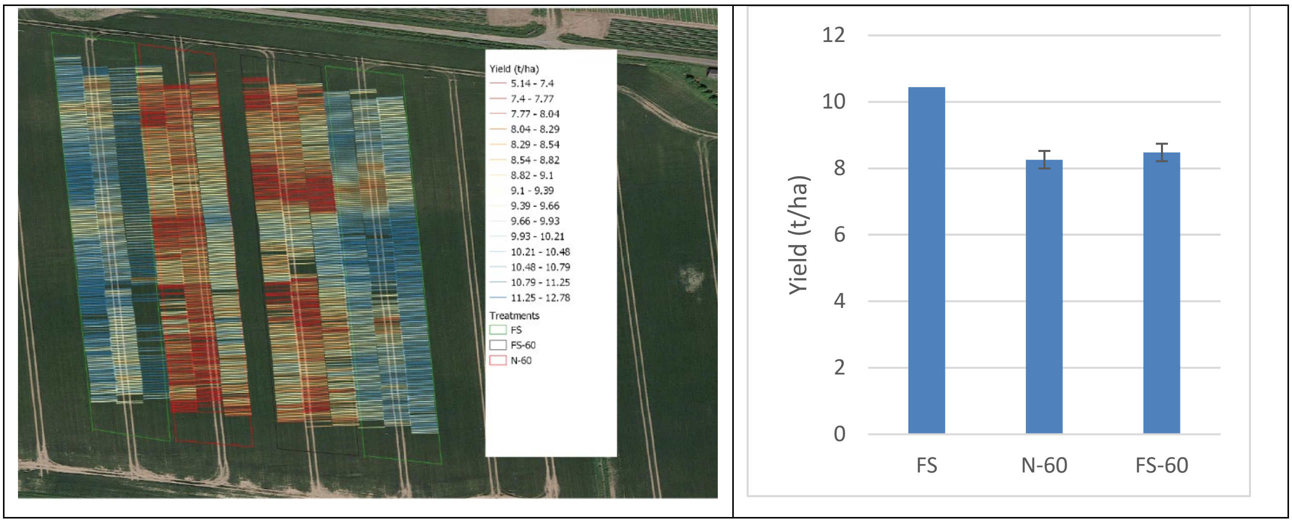

Figure 10. Strathmore winter wheat – modelled yield data from Agronomics analysis. Error bars represent SED.

The farm standard treatment was significantly higher yielding (P<0.001) than the other treatments at 10.44t/ha at 85%DM. The FS-60 treatment yielded 2.18t/ha less than the FS treatment and the N-60 treatment yielded 1.96t/ha less (Figure 10). There was no significant difference in yield between the FS-60 and N-60 treatments.

For the other three trials, yield data was obtained using a weighbridge. In the winter wheat trial at Grange of Conon, the N-40 treatment yielded 1.25t/ha less than the FS treatment which yielded 9.73t/ha (Table 7). In the spring barley trial at Strathmore, the Nurture + treatment resulted in a considerable yield increase of 1.12t/ha (Table 8). At Grange of Conon, the N-20 treatment resulted in a small yield increase of 0.1t/ha. Given that these yields were determined from weighbridges, it was not possible to perform statistical analyses to determine whether the effects were driven by the treatments or by possible underlying variation in the field.

Table 7 Grain yield (t/ha) from farm weighbridge (wheat)

	Farm Standard	N-40
Grange of Conon	9.73	8.48

Table 8 Grain yield (t/ha) from farm weighbridge (Spring barley)

	FS	N+
Strathmore	7.83	8.95
	FS	N-20
Grange of Conon	8.32	8.42

4.5. Yield components

To understand the nature of the yield effects observed in the winter wheat trials in more detail, components of yield were determined from quadrat samples collected approximately 2 weeks before harvest. The lower yield obtained with the FS-60 and N-60 treatments appeared to be driven by a significantly lower number of ears/m^2, resulting in significantly less grains/m^2 (Figure 11). Whilst the number of grains/ear was less for the FS-60 and N-60 treatments, this difference was small and not statistically significant. There was also a notable reduction in DMHI in both reduced N treatments, where the DMHI was reduced by 1.8 and 3.1% respectively for the FS-60 and N-60 treatments. Additionally, there was also a significant effect of the treatments on TGW, with a TGW of 50.4g for the FS treatment, 47.7g for the FS-60 treatment and 47.3g for the N-60 treatment.

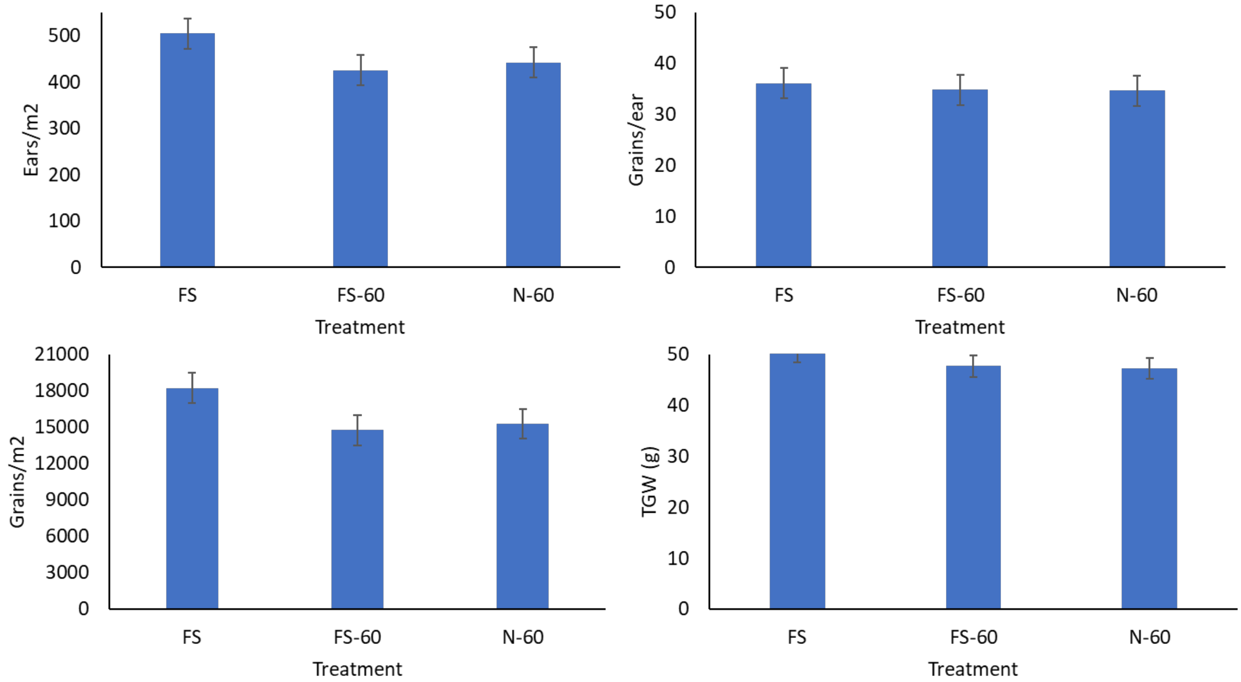

Figure 11. Strathmore winter wheat – yield components from pre-harvest samples. Error bars represent LSDs.

Analysis of quadrat samples taken from the winter wheat trial at Grange of Conon demonstrated that ears/m^2 was significantly reduced by the N-40 treatment, whilst there was no significant effect on grains/ear (Figure 12). The N-40 treatment resulted in a significantly lower number of grains/m^2, although there was no effect of the treatment on how well these grains were filled, with a TGW of 46g for both treatments.

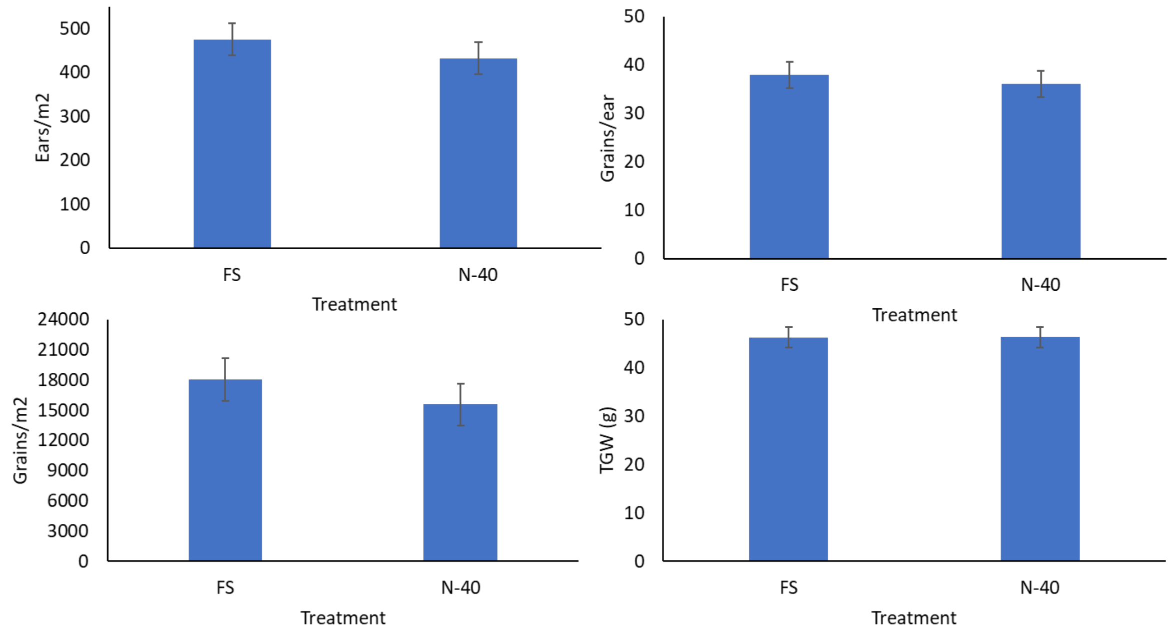

Figure 12. Grange of Conon winter wheat - yield components from pre-harvest samples. Error bars represent LSDs.

For the spring barley sites the number of ears were counted at mid grain filling in the field (Figure 13). Ear numbers were much greater at Grange of Conon than at Strathmore. There were no significant differences between treatments at either site. Therefore, ear numbers do not explain the apparent yield differences seen in the Strathmore experiment.

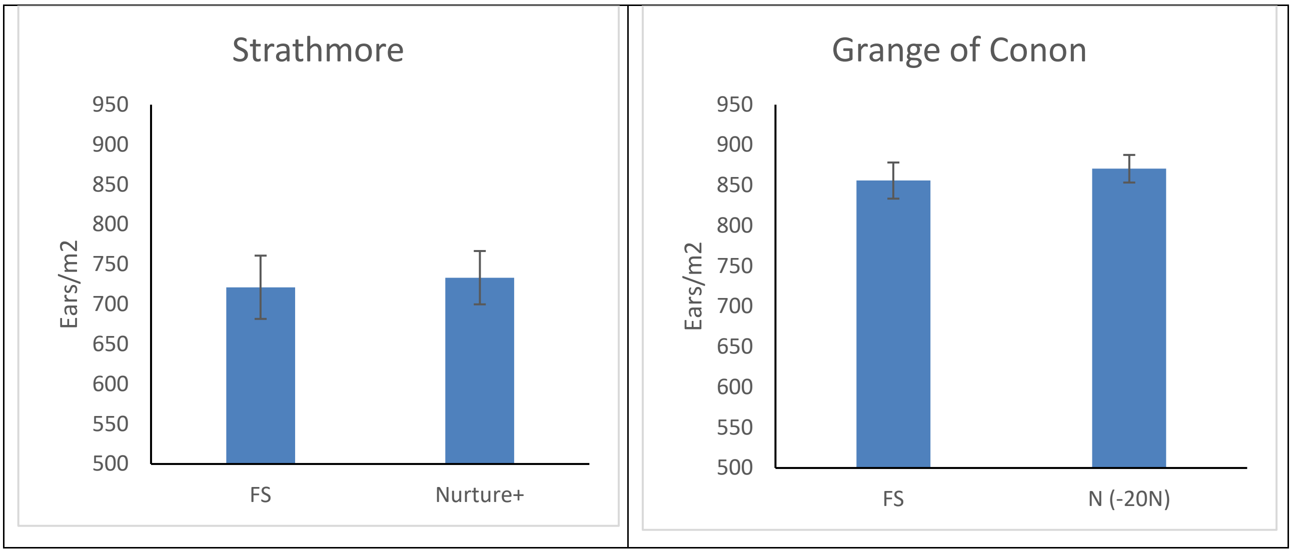

Figure 13. Grange of Conon winter wheat - yield components from pre-harvest samples. Error bars represent LSDs.

4.6. Grain nutrition

The use of grain analysis to measure the nutrients which are harvested has been used in the YEN since 2016, and a separate network; YEN Nutrition, now focusses on this. In the Strathmore winter wheat trial (Table 9), tissue analysis highlighted N as being slightly low in the FS and the FS-60 treatment whereas in the grain analysis, concentrations of N in the grain were considerably lower than the 1.93% YEN low value, for all treatments, suggesting that N may have limited yield in this crop. Reducing the N by 60kg N/ha reduced the grain N% further, whilst the application of Nurture N had very little effect on grain N%. Additionally, for all three treatments, grain P, S and B levels were on the low side and this was consistent with results from the tissue analysis. In contrast to the tissue analysis results which were low, the Mo concentration in the grain was adequate across the three treatments. The grain analysis results also highlighted that concentrations of Zn, Fe and Cu were also lower than the YEN low value.

Table 9. Grain Nutrition – Strathmore winter wheat trial

	N	P	K	S	Ca	Mg	B	Cu	Mn	Mo	Fe	Zn
	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
YEN (low)	1.93	0.27	0.37	0.12	0.04	0.08	0.8	3.4	21	0.29	34	20
FS	1.45	0.25	0.44	0.09	0.03	0.08	0.5	2.6	24	0.38	23	13
N-60	1.17	0.25	0.47	0.08	0.03	0.07	0.5	2.3	23	0.46	18	11
FS-60	1.19	0.21	0.43	0.08	0.02	0.07	0.43	2.4	19	0.35	19	10

Table 10. Grain nutrition Grange of Conon winter wheat trial

	N	P	K	S	Ca	Mg	B	Cu	Mn	Mo	Fe	Zn
	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
YEN (low)	1.93	0.27	0.37	0.12	0.04	0.08	0.8	3.4	21	0.29	34	20
FS	1.3	0.24	0.45	0.09	0.02	0.08	0.42	2.3	21.3	0.29	20.5	11.8
N-40	1.2	0.24	0.46	0.08	0.02	0.07	0.44	2.2	21.4	0.33	20.4	11.6

Similarly, for the winter wheat trial at Grange of Conon (Table 10), grain N% was also significantly lower than the 1.93% YEN low value, despite both treatments displaying adequate levels in the tissue analysis. Levels of B in the grain also appeared to be on the low side, a consistency with the tissue results. On the whole, levels of nutrients in the grain were generally similar between the two treatments. There were similarities when the grain analysis results for the spring barley trial at Grange of Conon were considered (Table 11), with grain N% low in both the FS and N-20 treatment. S, B, Cu, Zn showed lower concentrations than the YEN low value in both treatment situations, whilst concentrations of Mo were notably higher than the YEN low value.

Table 11. Grain nutrition Grange of Conon spring barley trial

	N	P	K	S	Ca	Mg	B	Cu	Mn	Mo	Fe	Zn
	%	%	%	%	%	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg
YEN (low)	1.7	0.28	0.43	0.12	0.05	0.1	0.8	3.9	13	0.38	44	23
FS	1.1	0.24	0.41	0.08	0.04	0.08	0.6	2.7	14	1.11	58	15
N-20	1.3	0.27	0.44	0.09	0.04	0.08	0.6	2.2	14	0.57	42	20

4.7. Grain Fatty Acids

Recently there has been considerable interest in the nutritional quality of grain in relation to fat intake. In humans these omega-3 fats have anti-inflammatory properties and a deficiency contributes to a wide range of adverse mental and physical health conditions, including coronary heart disease. However, a major weakness of many modern diets around the world is a shortage of these types of fatty acids, exacerbated by excess omega-6 fats.

For grain fed to animals there is also a clear link between what is fed to them and the quality of meat which consumers eat. Feeding grains and cereal by-products produces less omega-3 fatty acids in the meat and more of the less desirable omega-6 fatty acids than pasture fed animals. The ratio of the omega-6 to omega-3 is also much poorer for grain-fed beef at 7:1 compared to a healthier 2:1 for beef from 100% pasture-fed cattle (Butler et al 2021). Decreasing the omega-6 to omega-3 ratio in cattle feed will thus lead to healthier choices for consumers.

There is also no obvious effect of N reduction on grain fatty acid content as apparent reductions seen in the winter wheat contrasted to increases in the spring barley (Table 12). Samples analysed were unreplicated so it is not possible to draw any firm conclusions from this data. The omega-6 to omega-3 ratios (in whole sample) were 10:1 and 12:1 in winter wheat and 9.5:1 and 8:1 for farm standard and treated strips respectively. This data will however provide a baseline for further investigation by the farmers themselves.

Table 12. Grain fatty acid groups from Grange of Conon trials showing differences between samples. For calculation of differences values of <0.05 have been assumed to be 0.05. Full laboratory analysis results in appendix 1.

Fatty Acids	Units	WW FS	WW-40N	WW (diff)	SB-FS	SB-20N	SB (diff)
Unidentified Fatty Acids	% (of TFA)	3.63	3.93	-0.3	3.83	3.75	0.08
Saturated Fatty Acids	% (of TFA)	22.07	20.17	1.9	23.32	24.93	-1.61
Monounsaturated Acids	% (of TFA)	12.2	12.91	-0.71	13.65	12.74	0.91
Polyunsaturated Acids	% (of TFA)	62.22	63.08	-0.86	59.33	58.7	0.63
Omega 3 (in oil)	%	5.03	4.53	0.5	5.32	6.11	-0.79
Omega 3 (in sample)	%	0.08	0.09	-0.01	0.14	0.15	-0.01
Omega 6 (in oil)	%	54.45	55.77	-1.32	51.4	50.01	1.39
Omega 6 (in sample)	%	0.89	1.09	-0.2	1.36	1.26	0.1

5. Discussion

Analysis of the data from the four trials, along with the discussions at each of the four meetings held across the course of the project have highlighted a number of points for consideration in addressing the hypotheses set out by the farmers at the start of the project in the future.

The results from the winter wheat trials highlighted that significant yield reductions were obtained in response to reducing N rates by 60 kg N/ha and 40 kg N/ha at Strathmore and Grange of Conon (Figure 10 and Table 7). In the Strathmore trial, the application of Nurture N and additional nutrient and biostimulant products had no effect on yield in the reduced N situation in the trials. Physiologically, the reduced N rate resulted in a significantly lower number of ears/m^2 and grains/m^2 in both trials (Figures 11 and 12). Notably, at Strathmore the N-60 and FS-60 treatments resulted in a lower TGW, whereas there was no effect of the N-40 treatment at Grange of Conon.

In contrast to the winter wheat trials, there was minimal effect of the reduced N treatment with Nurture N applied at Grange of Conon in spring barley, providing an indication that the current FS application rate may be in excess and therefore there may be scope for reducing rates further. It should be considered that due to this being a weighbridge yield with no replication, it is not possible to understand the variability in this result. However, grain N% results from the trial showed that N was low in both treatments at 1.1% and 1.3% for the FS and N-20 treatments respectively, indicating that N may have limited yields (Table 11). In the Strathmore experiment, the results showed a large yield increase in excess of 1t/ha in response to the Nurture + treatment. This result should be treated with caution since there was no replication, and such a large yield increase in response to foliar nutrition and biostimulant products are not common in the literature (Roques et al., 2013; Storer et al., 2016). NDVI imagery (Figure 9) did not indicate increased NDVI in the Nurture + treatment, in fact the NDVI in this area looked to be lower than the FS treated area. Additionally, the assessment of ears/m^2 provided no indication of treatment effects and did not support the increase in yield. It is possible that other components of yield, such as grains/ear, grains/m^2 or TGW was increased in response to the Nurture + treatment, but these were not measured in these trials. It would be recommended that this trial should be repeated, with additional replication and physiological measurements to help explain effects on yield.

The grain nutrient analyses highlighted a range of nutrients which may need attention in future seasons across both farms (Tables 9 – 11). There was no clear evidence for the treatments resulting in substantially different concentrations of nutrients in the grain. Unfortunately, grain samples from the spring barley trial at Strathmore were not analysed, so it is not possible to determine if the products had resulted in different concentrations of nutrients. However, the tissue analysis showed a very similar picture for the FS and Nurture + treatments.

Kindred *et al*., (2018) highlighted the usefulness of farmers testing the effect of applying 60 kg N/ha less and more than their farm standard N rate in tramline trials to ascertain if their N rate was too high, too low or about right. With pressures to optimise N fertiliser use, as well as considerable interest in reducing N rates to minimise GHG emissions, such on-farm trials can help farmers to make informed decisions about their N fertiliser use. Recent work by Kendall *et al*., (2021) has resulted in updated recommendations for N and S use in spring barley, in which research concluded that current RB209 recommendations over estimate economic optimum N rates by over 40kg N/ha, and therefore, there is potential for reducing rates of N applied to spring barley which follow current recommendations without significant economical effects. Therefore, it is highly recommended that the farms involved in this project continue to perform on farm trials to understand how to optimise N use on their own farms.

The treatments had already been applied by the farmers before the onset of the project, and therefore it was not possible for the researchers and farmers to co-design the layouts. The importance of robust trial designs was discussed between the researchers and farmers during the meetings. These included: i) understanding intra-field variation and aiming to minimise the impact of this variation on the trial; ii) consideration of level of replication – increasing replication will improve the statistical power for the analysis of both weighbridge and yield map analyses and iii) the benefit of analysing yield map data where individual yield points improve the statistical power of the analysis. The farmers involved in the project would be able to take points from these discussions and apply them in establishing trials on their farms in the future.

Nuture N was applied after main N applications on each farm and the farmers agreed that this was later than ideal. It was agreed that it would have been more appropriate to test the ability of Nuture N to improve N uptake by the crop if it was applied at the same time as the N application. In some of the treatments, Nuture N was applied in combination with other biostimulant and micronutrient products so it is difficult to ascertain the effect of the range of products included. However, it should be recognised that testing an approach or system which includes multiple products can be a useful approach, and that testing individual products separately is not necessarily viable in a commercial setting. Additionally, in the two trials performed at Grange of Conon, there was no reduced N treatment without Nuture N, and therefore, it is difficult to disentangle the effect of the reduced N and the effect of Nuture N. An alternative approach would have been to include a separate reduced N treatment.

Above and beyond the trials themselves, a number of topics were discussed with researchers providing information where applicable and farmers outlining current and future needs and concerns for their businesses. Many of the discussions centred around improving nutrient use efficiency and grain quality. Having an organic farmer as part of the group was also particularly useful when talking about sustainability issues as it introduced a wider perspective than more focussed single topic discussions can achieve. Together, this project represented a successful partnerships between farmers and researchers to maximise the learnings from farmer-led on-farm trials.

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7. Appendix 1

Full results from the fatty acid analysis of grain from WW and SB trials at Grange of Conon.

Test	Fatty Acid	Units	WW FS	WW-40	SB-FS	SB-20
Oil	(Oil B) Acid	% (of TFA)	1.64	1.95	2.65	2.53
C08:0	Caprylic Acid	% (of TFA)	<0.05	0.06	0.05	<0.05
C10:0	Capric Acid	% (of TFA)	0.12	0.17	0.1	0.1
C11:0	Undecylic Acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C12:0	Lauric Acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C13:0	Tridecylic Acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C14:0	Myristic Acid	% (of TFA)	0.16	0.08	0.18	0.23
C14:1	Myristoleic Acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C15:0	Pentadecanoic Acid	% (of TFA)	0.09	0.1	0.09	0.1
C15:1	Pentadecenoic Acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C16:0	Palmitic Acid	% (of TFA)	20.05	18.45	20.57	22.27
C16:1	Palmitoleic Acid	% (of TFA)	0.14	0.14	0.14	0.15
C17:0	Heptadecanoic Acid	% (of TFA)	0.1	0.11	0.1	0.08
C17:1	Heptadecenoic Acid	% (of TFA)	0.08	0.11	0.09	0.06
C18:0	Stearic Acid	% (of TFA)	0.84	0.61	1.09	1.18
C18:1	Oleic Acid	% (of TFA)	11.23	12.04	12.42	11.47
C18:2	Linoleic Acid	% (of TFA)	56.84	58.25	53.64	52.19
C18:3	gamma Linolenic Acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C18:3	alpha Linoleic Acid	% (of TFA)	5.11	4.71	5.15	5.92
C18:3	Linolenic Acid	% (of TFA)	5.11	4.71	5.15	5.92
C18:4	Stearidonic Acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C20:0	Arachidic acid	% (of TFA)	0.15	0.12	0.2	0.21
C20:1	Gadoleic acid	% (of TFA)	0.61	0.5	0.79	0.83
C20:2	Eicosadienoic acid	% (of TFA)	0.12	0.09	0.13	0.12

C20:3	Dihomo-gamma-linolenic acid (DGLA)	% (of TFA)	0	0	0	0
C20:4	Arachidonic acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C20:3	Eicosatrienoic acid (ETE)	% (of TFA)	0	0	0	0.12
C22:0	Behenic acid	% (of TFA)	0.19	0.14	0.49	0.39
C20:5	Eicosapentaenoic acid	% (of TFA)	0.15	<0.05	0.41	0.39
C22:1	Erucic acid	% (of TFA)	0.11	0.09	0.17	0.19
C22:4	Adrenic acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C24:0	Lignoceric acid	% (of TFA)	0.2	0.19	0.28	0.21
C22:5	Docosapentaenoic acid	% (of TFA)	<0.05	<0.05	<0.05	<0.05
C22:6	Docosahexaenoic acid	% (of TFA)	<0.05	<0.05	<0.05	0.08
	Unidentified Fatty Acids	% (of TFA)	3.63	3.93	3.83	3.75
	Saturated Fatty Acids	% (of TFA)	22.07	20.17	23.32	24.93
	Monounsaturated Acids	% (of TFA)	12.2	12.91	13.65	12.74
	Polyunsaturated Acids	% (of TFA)	62.22	63.08	59.33	58.7
Estimated	Omega 3 (in oil)	%	5.03	4.53	5.32	6.11
Estimated	Omega 3 (in sample)	%	0.08	0.09	0.14	0.15
Estimated	Omega 6 (in oil)	%	54.45	55.77	51.4	50.01
Estimated	Omega 6 (in sample)	%	0.89	1.09	1.36	1.26