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Effect of Clover Sward Management on Nitrogen Fixation and Performance of Following Spring- and Winter Wheat Crops; Results of a 3-Year Pilot Study

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Abstract: Wheat yields in organic production are significantly lower than those achieved in conventional farming systems and in Northern Europe organic farmers also struggle to achieve the processing quality levels demanded by millers and bakers, especially in winter-wheat crops. Here, we report the findings of a 3-year pilot study which investigated the potential of increasing grain yields and both standard processing (e.g., grain protein levels and specific weights) and selected nutritional (tocopherol and tocotrienol isomers) quality parameters via (i) changes in the management of clover crops grown before wheat (*Rhizobium* seed inoculation, application of greenwaste compost to clover swards) and (ii) use of new varieties developed in both the UK and continental Europe. Results indicate that the use of compost amendment to preceding clover swards significantly increased the grain protein levels in the three winter-wheat, but not the four spring-wheat varieties, and also significantly increases grain yields in one winter-wheat variety (Greina). In contrast, the use of *Rhizobium*-inoculation was found to significantly reduce protein concentrations in the winter-, but not spring wheat varieties, and had no significant effect on grain yields in both winter and spring wheat. However, analysis of variance detected significant interactions between *Rhizobium* seed inocula in clover pre-crops and (a) compost application for grain specific weights in winter-wheat and grain hardness in spring wheat crops and (b) variety for grain protein content in spring wheat crops. No significant effect of clover pre-crop management on tocopherol and tocotrienol isomer profiles could be detected, although significant differences between varieties were detected in both winter and spring wheat. Results of this pilot study need to be confirmed in future trials, but indicate that both (a) changes in clover-management practices and (b) the selections of wheat varieties that are more suitable for organic farming systems has the potential to increase grain yield and allow organic farmers to more frequently achieve baking or milling grade quality.

Keywords: organic wheat; *Rhizobium* inoculum; clover management; greenwaste compost; N-fixation; grain yield; bread-making quality; tocopherol; tocotrienol

1. Introduction

Although organic cereal grains and products were reported to have a higher nutritional quality [1–5], previous studies also found that both grain yields and protein contents in organic wheat production systems are between 20% and 40% lower than those achieved in intensive conventional farming systems [5–9].

This is thought to be at partially due to (i) insufficient nitrogen (N) supply from fertilization regimes used by organic farmers and (ii) less efficient methods of crop protection (especially for *Septoria* and weed management) and (iii) poor adaptation of modern short straw wheat varieties, that were developed for conventional high mineral fertilizer input production systems, to organic farming conditions [6–11].

Growing wheat crops immediately after nitrogen-fixing grain or forage legume crops in the rotation can significantly increase N-supply to wheat crops in organic production systems [6–9,11,12]. Perennial grass-clover leys are therefore widely used in both mixed and stockless organic arable rotations in Northern Europe for both weed suppression and to increase soil N-levels [7,11,13,14]. In stockless, organic arable systems green manure crops are managed by repeatedly cutting and mulching (leaving cut plant material to de-compost on the soil surface) to improve N-availability [15]. However, this practice results in up to 40% of N being lost as gaseous emissions during the summer months [15–18]. For example, while gaseous N-losses from clover swards after the removal of a hay crop were reported to range from 1.3 to 4.7 kg N/ha, losses were found to be 8 times higher when clover was cut and mulched [17].

Rhizobium inoculation of legumes crops is a well-established method to increase N-fixation and productivity of grain legume crops, but has not been widely used in organic production systems for forage and fertility building ley crops [19–22]. This is mainly because organic producers believe that this would not be cost effective, because there is sufficient natural *Rhizobium* inoculum in organically managed soils for efficient root infection and the rapid establishment of N-fixing root nodules on newly established rotational grass-clover leys [6], Lawrence Woodward OBE, personal communication). However, this hypothesis has not been tested under Northern European conditions and the use of *Rhizobium* inoculum may still be of benefit, especially (i) when farms convert to organic management and establish grass/legume leys for the first-time during conversion, (ii) in field where legumes belonging to the same cross-inoculation group have not been grown for more than 5 years or (iii) when ley crops are established under adverse weather conditions for *Rhizobium* infection from soil [6,21].

High concentrations of water-soluble, readily plant available forms of nitrogen (NH_4^+, NO_3^-) in soils are known to reduce the establishment and/or N-fixation levels of legume root nodules [6]. There is also some evidence that agronomic interventions which increase the assimilation/immobilization of NH_4^+ and NO_3^- by the soil biota (e.g., the application of high C:N-ratio organic matter to legume leys) may also increase symbiotic N-fixation and/or reduce N-losses, especially in perennial leys crops [22,23].

Although a positive correlation between straw length and protein content in wheat grain have long been recognized in continental Europe (e.g., most high baking quality/protein A and E wheat varieties in the German variety list are longer straw varieties), the higher lodging risk from longer straw varieties under UK conventional farming conditions has resulted in the disappearance of these varieties from the UK national listing (Ref. [24], Lochow Petkus, Bergen, Germany and CPB Twyfords, Roysten, UK, personal communication). However, since the risk of lodging was found to be substantially lower in organic production systems in the UK [6,9] one strategy to increase the protein content was therefore thought to be the evaluation of longer-straw varieties from European breeding programmes under commercial organic farming conditions in Northern Britain.

The main objectitableve of the pilot factorial field experiment reported here was therefore to evaluate the effects of (i) inoculation of clover seed (grown as clover ley prior to wheat crops) with clover-specific *Rhizobium* seed inocula and (ii) applications of high C:N ratio greenwaste-based compost to cut clover green manure (designed to increase N availability for the following wheat crop) on the performance of selected winter- and spring-wheat varieties.

Winter-wheat varieties included in the pilot study were longer-straw cultivars from a Swiss/German organic farming-focused breeding programmes, while the spring-wheat varieties tested included modern short-straw varieties developed for the conventional farming sector in the UK and older, long-straw varieties developed in Germany and Sweden for the production of high-protein content, bread-making wheat.

2. Materials and Methods

2.1. Trial Site Characteristics and Soil Analyses

The site at Gilchesters Organic Farm, Northumberland UK (55°2′28.89″ N 1°53′28.73″ W; Supplementary Figure S1) comprised 24.3 ha of a 60 ha block of arable land, having been in continuous arable production for 15 years up to the harvest of 2002. The 24.3 ha comprised of four fields of roughly 6 ha each, managed as one complete arable block with the same conventional rotation across all 4 fields, of winter feed wheat, winter milling wheat, barley and oilseed rape. The crops were managed to conventional farming standards and received regular mineral N, P and K inputs according to ADAS recommendations. All straw, except for oilseed rape straw was removed from fields. Prior to the start of the experiment field had received no manure, compost or other organic matter inputs and fields had not been used for grass or clover leys for over 15 years.

Soil analyses of the top soil (0–30 cm) were carried out in all 4 field before the start of the experiment (15 January 2003) and sent to Glenside Fertility Farming Systems (Throsk, Stirling, UK) for analysis (Table 1).

Table 1. Results of soil analysis carried for the 4 fields used as blocks/replicates carried out before the start of the experiment in 2002.

Parameter Assessed	Field/Block Number			
	1	2	3	4
pH (H_2O)	6.0	6.3	6.7	6.9
N (kg/ha)	92	93	112	106
S (ppm)	10	9	12	16
P (kg/ha)	97	147	164	196
K (kg/ha)	133	175	250	557
Ca (kg/ha)	4879	5396	5505	4871
Mg (kg/ha)	628	776	679	948
Ca:Mg ratio	64	66	72	63
Na (kg/ha)	75	83	72	56
Fe (ppm)	533	665	722	908
Mn (ppm)	64	83	61	87
Cu (ppm)	1.7	2.0	1.9	1.3
Zn (ppm)	7.4	8.0	7.4	7.2
B (ppm)	0.50	0.53	0.80	1.08
Mo (ppm)	1.36	1.84	1.60	1.72

Further soil samples were taken after incorporation of clover leys in October 2005 in all variety sub-plots that were subsequently used for growing (i) the two winter wheat varieties Greina and Wenga and (ii) the two spring wheat varieties Paragon and Fasan. Samples were taken from both the top soil (0–30 cm) and subsoil (30–60 cm and 60–90 cm) and analyses were carried by Sabanci University, (Tuzla, Turkey) as part of the EU-collaborative project QualityLowInputFoods (https://cordis.europa.eu/project/id/506358/reporting, accessed 1 August 2022).

Mineral N and C were analyzed by Dumas Combustion and all other mineral nutrients by inductively coupled plasma optical emission spectroscopy (ICP-OES) as previously described [8,25].

Prior to the establishment of clover, the 24.3 ha site was aerated by subsoiling with a Kverneland CTC cultivator (Kverneland group, Klepp, Norway) post-harvest (September 2002) to a depth of 30–35 cm.

The site along with the rest of the farm entered into an organic conversion scheme in September 2002. The trial site was registered as "in-conversion" until September 2004, after which time it became fully certified for organic production. The impact of clover management on N fixation during the 2-year conversion period, while the experimental wheat crops were grown in the first growing season after the end of the conversion period.

2.2. Experimental Design and Agronomic Management

The experiment was established on 26 March 2003, on four 6 ha fields which were acting as replicate blocks. A factorial experimental design with (i) *Rhizobium* inoculation (+/−), (ii) high C:N ratio greenwaste compost amendment to clover swards (+/−) and (iii) wheat variety (4 spring and 3 winter wheat varieties) as factors was used (Figure 1).

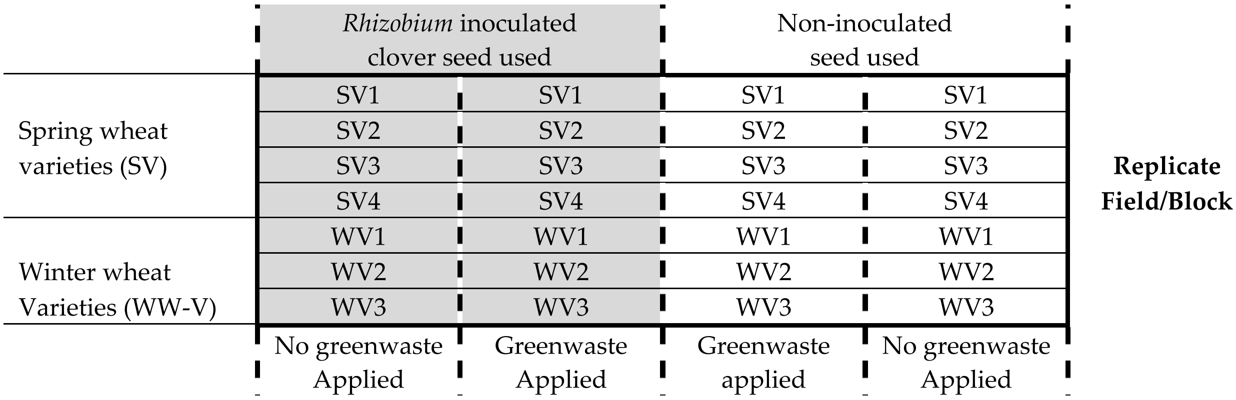

Figure 1. Field trial design *Rhizobium* inoculation main plots, greenwaste amendment subplots and variety sub-subplots in each replicate field/block.

This resulted in a 2 × 2 × 7 factorial split-split-plot experiment with 4 replicate blocks/fields and a total number of 112 plots (Figure 1). In each replicate field/block the position of *Rhizobium* inoculation main plots, greenwaste amendment sub-plots and variety sub-subplots was randomized. The experiment was used to assess (i) the impact of *Rhizobium* inoculum on the establishment and *Rhizobium* activity of the clover crop in year 1 (2003) and (2) of greenwaste compost (GWC) amendments in years 1 and 2 (2003–2004). The impact of both these treatments on the production and retention of N was assessed on the subsequent wheat variety trials (2005), which was the first organic wheat crop post conversion.

A pure red clover (*Trifolium pratense* L.) mix of 60% late flowering diploid (cultivar Britta) and 40% early tetraploid (cultivar Rotra) organic seed was purchased from a commercial seed merchant (McCreath Simpson & Prentice, Berwick-upon-Tweed, UK). Each field/replicate block was subdivided into 2 main plots each of 3 ha in which pure red-clover swards were established using seed that were either (i) untreated or (ii) inoculated with a commercial *Rhizobium* inocula only. The site was prepared with HE-VE Combi discs (HE-VA Doublet, Ørding, Denmark) and the clover was then sown using a Väderstad Rapid A400S direct drill (Väderstad Ltd., Grantham, UK) at a seed rate of 9.8 kg ha^{-1} and rolled. All the untreated plots were sown first, followed by sowing the treated seed on the same day (26 March 2003), to avoid cross-contamination of control plots with *Rhizobium* inoculum.

2.3. Clover Seed Treatment

For inoculation 2 kg of the commercial *Rhizobium* product (Becker-Underwood, Saskatoon, SK, Canada) were applied to 120 kg of clover seed. The inoculum was supplied in a finely ground peat-based carrier material and was mixed manually with the seed as recommended by the manufacturer. The commercial *Rhizobium* inoculum used is one of the strains isolated at Rothamstead Research Centre from those naturally occurring in UK soils, and was multiplied and cultured by Microbio Group Ltd. (Stockport, UK; a wholly owned subsidiary of Becker Underwood).

2.4. Clover Crop Management

Each main plot was then subdivided into 2 sub-plots of 1.4 to 1.7 ha, one of which was to receive no inputs while the other receives 15 t/ha of a high C:N greenwaste compost (supplied by COMVERT Ltd., Morpeth, UK). All plots were topped at the end of May/beginning June and again on 28–29 August 2003 and biomass left on the ground as a mulch. The greenwaste compost (GWC) was applied to each half sub-plot immediately after each topping of the clover. Compost composition was analyzed at NRM Laboratories (Natural Resource Management Ltd., Bracknell, UK) (Table 2). The same procedure for topping and GWC application was repeated in 2004.

Table 2. Composition of composted greenwaste (analytical results are expressed on a dry matter basis).

Dry Matter	40	%
Conductivity 1:6	812	uS cm
Total Nitrogen	1.54	% ww
Total Carbon	20.3	% ww
C:N ratio	13:1	
Nitrate Nitrogen	159	mg/kg
Ammonium Nitrogen	126	mg/kg
Total Phosphorus (P)	2583	mg/kg
Total Potassium (K)	5850	mg/kg
Total Copper (Cu)	23.1	mg/kg
Total Zinc (Zn)	143	mg/kg
pH	7.8	

2.5. Clover and Rhizobium Nodule Assessments

Each of the 112 plots and sub-plots was assessed aboveground for clover plant numbers (to determine % establishment) and below ground for *Rhizobium* development. Assessments were made and samples taken during each of the growing seasons, in 2003 (Year 1) and in 2004 (Year 2). In Year 1 the first samples were taken between 15–25 May 2003, prior to the first clover topping and the first GWC application. In year 2 sampling was done post topping and GWC applications.

Due to the scale of the site and the size of each plot and sub-plot, the location of sampling stations was determined by a combination of random and stratified sampling techniques. The stratified technique required calculating the length of the diagonal of each plot and dividing by 6. This gave 5 sampling station locations per plot along the diagonal of each plot excluding the margins. The randomization technique was to select the distance of the sample stations from 1 to 5 m off the diagonal by a simple roll of a dice and then alternatively left or right of the diagonal. Sample stations measured 20 cm^2, recording the number of clover plants within the quadrat to determine the level of establishment (plants per m^2).

For *Rhizobium* nodule assessments, the root system from one plant, chosen at random from within each sample station was laid out over a 16 × 12 cm grid of 12 × 4 cm^2 squares and analyzed for: number of *Rhizobia* nodules, location of nodules on the root, active or inactive nodules and nodule volume/size. Nodule volume was calculated by measuring nodule length, width and height using a standard digital calliper (Oxford Precision OXD-331-2060K) applied to the formula for an oblate ellipsoid, given by $\frac{\pi}{6}.L.W.H$ cubic units (mm^3). Each nodule was then dissected to establish whether it was active or not. Nodules were recorded as active if a mottled pinkish interior was visible to the naked eye after cutting the nodule in half.

2.6. Wheat Varieties Used

On 28 October 2004 three bread-making winter wheat (*Triticum aestivum* L.) varieties and on the 9 March 2005 four bread-making spring wheat (*Triticum aestivum* L.) varieties were planted/superimposed on the fertility management plots (sub-sub-plots). Crops were sown using an Accord 3 m combination drill consisting of a Rabe power-harrow (Rabe

Werk, Bad Essen, Germany) and an Accord 24 row precision drill (Heinrich Weiste & Co., GmbH, Soest, Germany).

Winter wheats were sown at a density of 400 plants m^{-2} according to thousand grain weight (equivalent rate of 185 kg ha^{-1}), while spring wheats were sown at a density of 350 plants m^{-2} according to thousand grain weight (equivalent rate of 210 kg ha^{-1}). The varieties used/compared were:

- Greina, a short-straw winter wheat variety developed for the conventional farming sector by Agroscope/DSP as part of the Swiss national breeding programme,
- Wenga and Pollux, two long-straw winter wheat varieties from an organic farming focused breeding programme developed by Peter Kunz for Sativa (Reinau, Switzerland),
- Paragon and Monsun two short-straw spring wheat varieties developed by CPB-Twyford/KWS UK Ltd. (Roysten, UK) and Lochow Petkus (Bergen, Germany), respectively, for intensive conventional farming systems
- Fasan and Zebra, two long-straw spring wheat varieties developed for high baking quality bread wheat production by Lochow Petkus and Svalöf Weibull AB/Lantmännen Seed (Malmö, Sweden (Sweden) respectively.

2.7. Wheat Crop Assessments

Relative leaf chlorophyll or nitrogen activity in all wheat varieties across all treatments was recorded by single-photon avalanche diode detection (SPAD) assessed with a Minolta chlorophyll meter model SPAD 502 (Spectrum Technologies Inc., Plainfield, IL, USA), with 4 readings taken for each variety/treatment. Each reading was based on 10 leaf sample points. All readings were taken on 27 June 2005, at growth stage 55 (GS 55) for the winter wheat varieties and at GS 37 for the spring wheat varieties [26].

All standard grain analyses (protein content, Hagberg Falling Number, specific weight, grain hardness) were carried out at Campden BRI (Chipping Campden, UK) as part of the EU-collaborative project QualityLowInputFoods using standard protocols (www.campdenbri.co.uk/services/cereals-testing.php, accessed 1 August 2022). Briefly, to determine the dry matter content a subsample of grain was dried at 70 °C in an oven and then milled into fine powder (<1 mm) using a Retsch centrifuge mill (Retsch. GmbH, DE; www.retsch.com, accessed 1 August 2022). Flour N-content was determined with the Dumas combustion method by using a vario 210 MACRO cube C/N Analyzer (Elementar LTD, Germany) and grain crude protein concentration was calculated by multiplying N with 6.25. The Hagberg Falling Number was determined using a Perten Falling Number 1310 Analyser (PerkinElmer, Waltham, MA, USA). The grain Hardness Index/score is a measurement of the force required to crush each kernel and was determined using the Perten Single Kernel Characterisation System (SKCS) 4100 (Perten Instruments, Springfield, MA, USA).

Grain samples for tocopherol analyses were freeze-dried immediately after harvest by Newcastle University. Concentrations of α-Tocopherol, α-Tocotrienol, β-Tocopherol and β-Tocotrienol were assessed by the Food Analysis Department of the Central Food Research Institute of Hungary (CFRI, Herman Otto Vt 15, Budapest, Hungary) as part of the EU-collaborative project QualityLowInputFoods using a standard HPLC protocol. Vitamin E concentrations in grains were assessed as an important nutritional quality parameter in cereal grains. It is important to note that Vitamin E comprises four tocopherol isomers (alpha, beta, gamma and delta) and four tocotrienols isomers (alpha, beta, gamma and delta), but that α-tocopherol has the highest biological activity and is preferentially absorbed and accumulated in the human body. In this study grains were analyzed for α- and β-tocopherols and α- and β-tocotrienols only.

Crop yields were assessed by combining a 320 m^2 section from each treatment using a CLAAS Dominator 38 plot-combine harvester (CLASS, Harsewinkel, Germany). Grain fresh weights were determined by weighing grain harvested in each plot immediately after harvest. A sample of harvested grain was dried at 80 °C for two days using a drying oven (Genlab Ltd., Widnes, UK) and grain yields are presented at 15% moisture content. Protein analyses were carried out by the wheat breeding/seed production company Lochow Petkus GmbH (Bergen, Germany) using standard protocols (ICC Standard No. 159; ICC 2006). The dates of clover incorporation, soil analyses, and planting, mechanical weeding, chlorophyll assessments and harvest of wheat crops are shown in Table 3.

Table 3. Dates of soil incoporation of clover, planting of wheat crops mechanical weed control, assessments and harvest of winter and spring wheat varieties.

	Winter Wheat Varieties	Spring Wheat Varieties
Soil incorporation of clover ley	28 October 2004	28 October 2005
Planting of wheat crops	28 October 2004	09 March 2005
Soil Analysis	19 January 2005	19 January 2005
Weeding 1	04 April 2005	25 May 2005
Weeding 2		03 June 2005
Chlorophyll SPAD	27 June 2005	27 June 2005
Harvest	17 August 2005	07 September 2005

2.8. Statistical Analysis

For the 2003 and 2004 clover assessment data analyses of variance were derived from linear mixed-effects models [27] from data with a negative binomial distribution (non-symmetrical–due to the clumped nature of the samples recorded). Fixed effects were season, clover seed treatment, and fertility management, with fields as random effect, given the nested structure of the trial [28], and applied where necessary. For the 2004-05 data collected in spring and winter wheat plots, variety was added as additional fixed factor.

The effects of the fixed factors on chlorophyll content, yield, yield components, protein, hectolitre weight, hardness and Vitamin E levels in wheat were determined. Analyses of variance derived from general linear models and linear mixed-effects models were carried out in the R statistical environment (R-foundation [29]) and residual normality was assessed using the qqnorm function in R [29]. For significant interaction between factors, interaction means were determined and compared using Tukey's Honest Significant Difference (HSD) tests, based on mixed-effects models in R [29].

3. Results

3.1. Effect of Clover Management (Rhizobium Seed Inoculation and Greenwaste Compost Amendments) on Clover Nodulation and Performance Parameters

When clover plots were assessed in the first growing season (2003), Rhizobium inoculation was found to result in a significant increase in (i) clover plant establishment (number of plants/m^2), (ii) the numbers of *Rhizobium* root nodules/plant and (iii) the mean *Rhizobium* root nodule volume, while the proportion of active nodules was similar in clover plots established with inoculated and non-inoculated seeds (Table 4).

Table 4. Effect of *Rhizobium* inoculation of clover seed on clover establishment, nodule number/plant, % active nodules and mean nodule volume in growing season 1 (2003). Values shown are main effect means ± SE.

Factor	Clover Establishment (plants/m^2)	Nodulation (No/plant)	% Active Nodules	Mean Nodule Volume (mm^3)
***Rhizobium* seed inoculation**				
with	7.7 ± 0.1	149.1 ± 0.2	0.7 ± 0.08	3.9 ± 0.2
Without	6.2 ± 0.1	115.3 ± 0.1	0.73 ± 0.1	2.5 ± 0.1
1-factor ANOVA-results				
Main effect (*p*-value)	0.0217	0.0319	NS	0.0012

NS, not significant

In contrast, in the second growing season (2004), no significant effects of inoculum and greenwaste compost application were detected for root fresh weight, root nodule numbers per plant and mean root nodule volume (Table 5).

Table 5. Effect of *Rhizobium* inoculation of clover seed and greenwaste compost applications to clover swards on the mean root weight of clover plants, nodule number/plant and mean nodule volume in growing season 2 (2004). Values shown are main effect means ± SE.

Factors	Mean Root Fresh Weight (g)	Nodulation (No/plant)	Mean Nodule Volume (mm^3)
***Rhizobium* seed inoculation**			
with	32 ± 3	393 ± 31	1.1 ± 0.5
without	38 ± 6	356 ± 20	1.1 ± 0.3
Greenwaste compost application			
With	38 ± 5	333 ± 61	1.1 ± 0.3
Without	33 ± 2	417 ± 35	1.1 ± 0.5
2-factor ANOVA-results (*p*-values)			
Main effects			
Rhizobium seed inoculation (RI)	NS	NS	NS
Greenwaste compost application (GA)	NS	NS	NS
***Interaction** (RI × GA)*	NS	NS	NS

NS, not significant

It is also important to point out that nodulation increased, while the mean nodule volume decreased between assessment in year 1 and 2 (Tables 4 and 5).

Results from the soil analyses carried out in October 2004, before planting of winter wheat crops showed that, as expected, the total C, N, P and K content of soils was highest in the top soil (0–30 cm) and decreased with soil depth (0–30 cm > 30–60 cm > 60–90 cm) (Supplementary Tables S1 and S2). However, ANOVA detected no significant effects of Rhizobium inoculation and greenwaste applications on the total C, N, P and K and available N concentration in soils, although it should be noted that numerically both Rhizobium inoculation and greenwaste applications increased the available N concentrations by ~35% (Supplementary Table S1).

3.2. Effect of Clover Management (Rhizobium Seed Inoculation and Greenwaste Compost and Variety Choice on Grain Yield and Quality of Following Wheat Crops)

3.2.1. Winter Wheat Performance

When effect of clover management practices (*Rhizobium* seed inoculation, green-waste compost application) and variety choice on growth/performance parameters of winter wheat crops was assessed, 3-factor ANOVA detected significant main effects of (i) clover seed inoculation on leaf chlorophyl levels (SPAD) and the number of tillers at harvest, (ii) greenwaste compost on leaf chlorophyl levels and (iii) variety choice on the number of tillers at harvest and grain yield (Table 6).

Specifically, both *Rhizobium* seed inoculation and greenwaste compost applications significantly increased leaf chlorophyll levels and *Rhizobium* seed inoculation also significnatly increased the No of tillers at harvest (Table 6). The varieties Greina and Pollux had significantly higher leaf chlorophyll levels than the variety Wenga (Table 6). The highest grain yield was recorded for the variety Pollux followed by Greina and Wenga, which had a significantly lower grain yield than Pollux (Table 6).

When standard milling and breadmaking/baking quality parameters were assessed, all three winter varieties produced protein concentrations, Hagberg Falling Numbers (HFN), and specific weights (SW) required for baking or milling grade quality expected by UK organic millers (HGCA classification UKp, bread making, 11–13% protein, >250 s HFN and >76 kg/hl specific weight) (Table 7).

Table 6. Effect of clover ley management (*Rhizobium* inoculation of clover seed and greenwaste compost applications) on performance parameters (estimated leaf chlorophyll levels at growth stage 55, tiller numbers at harvest, grain yield) in contrasting winter wheat varieties established after two-year clover leys. Values shown are main effect means ± SE.

Factors	Leaf Chlorophyll at GS55 (SPAD)	Tillers at Harvest (ears/m^2)	Grain Yield (t/ha)
***Rhizobium* seed inoculation** [1]			
with	44 ± 1	302 ± 15	6.3 ± 0.2
without	43 ± 1	268 ± 18	5.6 ± 0.4
Greenwaste compost application [2]			
With	44 ± 1	297 ± 17	6.1 ± 0.4
Without	43 ± 1	276 ± 17	5.8 ± 0.2
Variety choice			
Greina	41 ± 2 b	319 ± 25 a	5.8 ± 0.5 ab
Pollux	42 ± 1 b	305 ± 14 a	6.4 ± 0.2 a
Wenga	49 ± 1 a	232 ± 15 b	5.7 ± 0.3 b
3-factor ANOVA-results (*p*-values)			
Main effects			
Rhizobium seed inoculation	0.0329	0.0073	NS
Greenwaste compost application (GA)	0.0460	NS	NS
Variety choice	T	0.0099	<0.0001
Interactions	3-factor ANOVA detected no significant interactions		

Variety main effect means labelled with the same letter within the same column are not significant different (Tukey's honestly significant difference test, $p < 0.05$); NS, not significant; T, trend ($0.01 > p > 0.05$); [1], of seeds used for preceeding clover crops; [2], to established preceding clover crops.

Table 7. Effect of clover ley management (*Rhizobium* inoculation of clover seed and greenwaste compost applications) on grain quality performance parameters (protein concentration, Hagberg falling number, specific weight and grain hardness) in contrasting winter wheat varieties established after two-year clover leys. Values shown are main effect means ± SE.

Factors	Protein (%)	Hagberg Falling Number	Specific Weight (kg/hl)	Grain Hardness (Ha)
***Rhizobium* seed inoculation** [1]				
with	11.7 ± 0.2	309 ± 11	82.1 ± 0.7	59.0 ± 2.5
without	12.5 ± 0.2	324 ± 12	80.5 ± 0.6	69.0 ± 2.0
Greenwaste compost application [2]				
With	12.4 ± 0.2	318 ± 13	81.3 ± 0.8	65.4 ± 2.9
Without	11.8 ± 0.2	315 ± 11	81.4 ± 0.6	62.4 ± 2.2
Variety choice				
Greina	11.9 ± 0.3	313 ± 17	81.9 ± 1.0	63.5 ± 3.7
Pollux	12.3 ± 0.2	320 ± 13	81.1 ± 0.8	64.3 ± 3.0
Wenga	12.1 ± 0.3	316 ± 13	81.0 ± 0.7	63.7 ± 2.7
3-factor ANOVA-results (*p*-values)				
Main effects				
Rhizobium seed inoculation (RI)	0.0179	NS	NS	NS
Greenwaste compost application (GA)	0.0140	NS	NS	NS
Variety choice (VC)	NS	NS	NS	NS
Interactions [3]				
GA × VC	NS	NS	0.0052	NS

NS, not significant; [1], of seeds used for preceeding clover crops; [2], to established preceeding clover crops; [3], only interaction which were significant for at least one parameter are shown.

Rhizobium seed inoculation resulted in significantly lower, while greenwaste compost applications resulted in slightly higher protein concentrations in wheat grain but, here were no significant main effects of clover management and variety choice on HFN, SW and grain hardness (Table 7). ANOVA also detected a significant interaction between greenwaste application to preceding clover crops and variety choice for the specific weight (SW) of grain (Table 7). When this interaction was further investigated compost amendment was found to significantly increase the SW of Greina, while there was no effect of compost amendment on the SW of Pollux and Wenga grain (individual interacton means not shown). It is interesting to note that 2-factor ANOVA (with *Rhizobium* inoculation and compost amendment as factors) of grain yield data for Greina found that compost applications to clover swards also results in significantly higher grain yields in Greina, while 2-factor ANOVAs for the other two varieties detected no significant main effect of compost and *Rhizobium* inoculation on grain yields (individual results not shown).

When grain concentrations of tocopherol and tocotrienol isomer were compared variety, but not clover management had a significant effect on isomer profiles (Table 8). For example, Greina had the highest β-tocopherol and lowest β-tocotrienol concentrations, Pollux had the highest α-tocopherol and lowest β-tocopherol concentrations, and Wenga had the highest α-tocotrienol and β-tocotrienol and the lowest β-tocopherol concentration (Table 8).

Table 8. Effect of clover ley management (*Rhizobium* inoculation of clover seed and greenwaste compost applications) on Vitamin E (tocopherol and tocotrienol isomers) concentrations (µg/g) in grain from contrasting winter wheat varieties established after two-year clover leys. Values shown are main effect means ± SE.

Factor	Vitamin E (Tocopherol/Tocotrienol) Isomers Monitored			
	α-Tocopherol	**α-Tocotrienol**	**β-Tocopherol**	**β-Tocotrienol**
***Rhizobium* seed inoculation [1]**				
with	12.1 ± 0.3	2.7 ± 0.1	6.2±0.2	21.5 ± 0.8
without	12.6 ± 0.3	2.9 ± 0.2	6.3±0.2	21.9 ± 0.8
Greenwaste compost application [2]				
With	12.4 ± 0.3	2.8 ± 0.1	6.2±0.2	21.5 ± 0.9
Without	12.3 ± 0.3	2.9 ± 0.1	6.3±0.2	21.9 ± 0.7
Variety choice				
Greina	11.5 ± 0.3 b	2.6 ± 0.1 b	6.9±0.2 a	17.9 ± 0.5 c
Pollux	13.0 ± 0.3 a	2.8 ± 0.1 b	6.2±0.2 b	22.4 ± 0.5 b
Wenga	12.5 ± 0.4 ab	3.0 ± 0.1 a	5.7±0.3 b	24.7 ± 0.9 a
3-factor ANOVA-results (*p*-values)				
Main effects				
Rhizobium seed inoculation	NS	NS	NS	NS
Greenwaste compost application	NS	NS	NS	NS
Variety choice	**0.0299**	**0.0159**	**0.0009**	**<0.0001**
Interactions	3-factor ANOVA detected no significant interactions			

Variety main effect means labelled with the same letter within the same column are not significant different (Tukey's honestly significant difference test, $p < 0.05$); NS, not significant; [1], of seeds used for preceeding clover crops; [2], to established preceding clover crops.

Overall, the total concentration of tocopherol and tocotrienol isomers assessed were higher in Pollux and Wenga than Greina grain.

3.2.2. Spring Wheat Performance

When effect of clover management practices (*Rhizobium* seed inoculation, greenwaste compost application) and variety choice on growth/performance parameters of spring wheat crops was assessed, 3-factor ANOVA detected significant main effects only for variety choice (Tables 9–11).

The highest grain yields were achived by the long-straw varieties Fasan and Zebra, although yields of the short straw variety Monsun were not significnatly different to those produced by Fasan (Table 9). In contrast, the short straw varieties Paragon and Monsun had higher leaf chlorophyll levels and tiller numbers at harvest, although tiller numbers produced by the long-straw variety Fasan were not significnatly different to those recorded for Paragon and Monsun (Table 9).

Table 9. Effect of clover ley management (*Rhizobium* inoculation of clover seed and greenwaste compost applications) on performance parameters (estimated leaf chlorophyll levels at growth stage 37, tiller numbers at harvest, grain yield) in contrasting spring wheat varieties established after two year clover leys. Values shown are main effect means ± SE.

Factors	Leaf Chlorophyll at GS37 (SPAD)	Tillers at Harvest (ears/m^2)	Grain Yield (t/ha)
***Rhizobium* seed inoculation** [1]			
with	45.6 ± 0.5	368 ± 12	7.0 ± 0.2
without	47.0 ± 0.5	339 ± 10	6.8 ± 0.2
Greenwaste compost application [2]			
With	46.1 ± 0.5	352 ± 13	6.9 ± 0.2
Without	46.5 ± 0.5	356 ± 9	6.9 ± 0.1
Variety choice			
Paragon (short straw)	47.4 ± 0.6 a	388 ± 16 a	5.7 ± 0.1 c
Monsun (short straw)	47.6 ± 0.6 a	355 ± 12 a	6.9 ± 0.2 b
Fasan (long straw)	45.0 ± 0.6 b	355 ± 17 a	7.0 ± 0.3 b
Zebra (long straw)	45.3 ± 0.7 b	317 ± 13 b	8.0 ± 0.3 a
3-factor ANOVA-results (*p*-values)			
Main effects			
Rhizobium seed inoculation	T	NS	NS
Greenwaste compost application	NS	NS	NS
Variety choice	<0.0001	0.0083	0.0008
Interactions	3-factor ANOVA detected no significant interactions		

Variety main effect means labelled with the same letter within the same column are not significant different (Tukey's honestly significant difference test, $p < 0.05$); NS, not significant; T, trend ($0.01 > p > 0.05$). [1], of seeds used for preceding clover crops; [2], to established preceding clover crops.

Table 10. Effect of clover ley management (*Rhizobium* inoculation of clover seed and greenwaste compost applications) on grain quality performance parameters (protein concentration, Hagberg falling number, specific weight and grain hardness) in contrasting spring wheat varieties established after two year clover leys. Values shown are main effect means ± SE.

Factors	Protein (%)	Hagberg Falling Number	Specific Weight (kg/hl)	Grain Hardness (Ha)
***Rhizobium* seed inoculation** [1]				
with	11.8 ± 0.1	354 ± 5	79.7 ± 0.4	68 ± 2
without	11.8 ± 0.1	361 ± 5	79.7 ± 0.5	67 ± 2
Greenwaste compost application [2]				
With	11.9 ± 0.1	361 ± 5	79.7 ± 0.5	69 ± 2
Without	11.7 ± 0.1	354 ± 6	79.7 ± 0.4	66 ± 2
Variety choice				
Paragon (short straw)	12.0 ± 0.2 ab	330 ± 5 b	78.1 ± 0.4 c	57 ± 2 c
Monsun (short straw)	12.1 ± 0.2 a	371 ± 7 a	77.9 ± 0.5 c	73 ± 3 a
Fasan (long straw)	11.7 ± 0.2 bc	368 ± 4 a	80.8 ± 0.3 b	68 ± 2 b
Zebra (long straw)	11.4 ± 0.2 c	362 ± 8 a	82.1 ± 0.6 a	72 ± 2 a
3-factor ANOVA-results (*p*-values)				
Main effects				
Rhizobium seed inoculation (RI)	NS	NS	NS	NS
Greenwaste compost application (GA)	NS	NS	NS	NS
Variety choice (VC)	0.0040	<0.0001	<0.0001	<0.0001
Interactions [3]				
RI × GA	NS	NS	NS	0.0253
RI × VC	0.0427	NS	NS	NS

Variety main effect means labelled with the same letter within the same column are not significant different (Tukey's honestly significant difference test, $p < 0.05$); NS, not significant; T, trend ($0.01 > p > 0.05$); [1], of seeds used for preceding clover crops; [2], to established preceding clover crops; [3], only interaction which were significant for at least one parameter are shown.

Table 11. Interaction means for effect of *Rhizobium* inoculation of clover seed and compost application to clover swards on the hardness of wheat grain grown after clover swards.

Factor 2	Factor 1	
Compost application	***Rhizobium*** **inoculation of clover seed**	
to clover swards	without	With
Without	68.1 ± 3.0 A a	64.7 ± 2.6 A b
With	65.5 ± 3.0 B a	71.7 ± 2.4 A a

Means with the same capital letters within rows and lower case letter within columns are not significantly different according to Tukey's Honest Significant Difference test ($p < 0.05$).

When standard milling and breadmaking/baking quality parameters were assessed all four spring wheat varieties achieved baking or milling grade quality sufficient for the requirements of current UK organic millers and for (HGCA, 2017). However, there were small, but significant differences in grain processing quality between the four varieties. (Table 10).

Specifically, (a) protein levels were highest in Monsun and lowest in Zebra grain, (b) the HFN of Paragon grain was significantly higher than that of the other three varieties, (c) the specific weight was highest in Zebra and lowest in the two short-straw varieties and (d) Grain hardness was highest in Monsun and Zebra and lowest in Paragon (Table 10).

ANOVA also detected significant 2-way interactions between (a) *Rhizobium* inoculation of cover seed and compost applications to clover swards for grain hardness and (b) in was *Rhizobium* inoculation of cover seed and spring wheat variety for grain protein content (Table 10). The use of *Rhizobium* inoculated clover seeds resulted in higher grain hardness when wheat was grown in plots where compost had been applied in the preceding clover swards, but lower grain hardness in plots that did not receive compost applications, although the effect of *Rhizobium* inoculum was only significant in compost fertilized plots (Table 11).

When the protein content produced by different varieties was compared in wheat grown after clover swards established with untreated seeds, Fasan and Monsun grain had a significantly higher protein content than Zebra grain. In contrast, when wheat grown after clover swards established with *Rhizobium* inoculated seeds was compared, Monsun and Paragon grain had a significantly higher protein content than Fasan and Zebra (Table 12).

Table 12. Interaction means for effect of *Rhizobium* inoculation of clover seed and wheat variety on the concentration of protein (%) in wheat grain grown after clover swards.

	Factor 1	
Factor 2	***Rhizobium*** **Inoculation of Clover Seed**	
Variety	Without	With
Fasan	12.0 ± 0.3 A a	11.3 ± 0.2 B b
Monsun	12.0 ± 0.3 A a	12.2 ± 0.3 A a
Paragon	11.9 ± 0.2 A ab	12.0 ± 0.2 A a
Zebra	11.2 ± 0.2 A b	11.6 ± 0.2 A b

Means with the same capital letters within rows and lower case letter within columns are not significantly different according to Tukey's Honest Significant Difference test ($p < 0.05$).

Similar to winter wheat, clover management had no significant effect on tocopherol and tocotrienol isomer in spring wheat crops, while significant differences in isomer profiles were detected between spring-wheat varieties (Table 13). For example, Paragon and Zebra had significantly higher α-tocopherol and β-tocopherol concentrations than Monsun and Fasan, Mosun had the highest and Fasan the lowest α-tocotrienol concentrations, and Paragon had the highest and Fasan the lowest β-tocotrienol concentrations (Table 13).

Table 13. Effect of clover ley managerment (*Rhizobium* inoculation of clover seed and greenwaste compost applications) on Vitamin E (tocopherol and tocotrienol isomers) concentrations (μg g^{-1}) in grain from contrasting spring wheat varieties established after two year clover leys. Values shown are main effect means ± SE.

Factor	**Vitamin E (Tocopherol/Tocotrienol) Isomers Monitored**			
	α-Tocopherol	**α-Tocotrienol**	**β-Tocopherol**	**β-Tocotrienol**
***Rhizobium* seed inoculation** [1]				
with	11.6 ± 0.5	2.6 ± 0.2	4.1 ± 0.21	19.0 ± 0.9
without	11.6 ± 0.5	2.9 ± 0.3	4.3 ± 0.21	18.7 ± 0.8
Greenwaste compost application [2]				
With	11.9 ± 0.5	2.6 ± 0.1	4.2 ± 0.21	18.5 ± 0.8
Without	11.3 ± 0.6	2.9 ± 0.3	4.2 ± 0.21	19.3 ± 0.8
Variety choice				
Paragon	12.8 ± 1.2 a	2.4 ± 0.2 bc	5.1 ± 0.2 a	22.36 ± 1.2 a
Monsun	10.7 ± 0.5 b	3.7 ± 0.5 a	3.0 ± 0.2 c	18.33 ± 1.1 b
Fasan	10.2 ± 0.2 b	2.0 ± 0.1 c	3.5 ± 0.1 b	14.82 ± 0.5 c
Zebra	12.7 ± 0.4 a	2.9 ± 0.2 b	5.1 ± 0.2 a	19.92 ± 0.7 b
3-factor ANOVA-results (*p*-values)				
Main effects				
Rhizobium seed inoculation (RI)	NS	NS	NS	NS
Greenwaste compost application (GA)	NS	NS	NS	NS
Variety choice (VC)	0.0199	0.0026	<0.0001	<0.0001
Interactions	3-factor ANOVA detected no significant interactions			

Variety main effect means labelled with the same letter within the same column are not significant different (Tukey's honestly significant difference test, $p < 0.05$); NS, not significant; ($0.01 > p > 0.05$), [1], of seeds used for preceeding clover crops; [2], to established preceeding clover crops.

Overall, total concentration of the tocopherol and tocotrienol isomer assessed was higher in Paragon and Zebra than Monsun and Fasan.

4. Discussion

4.1. Yield and Processing Quality

Previous studies suggested that it is difficult to achieve baking or milling grade quality (HGCA classification UKp, bread making, 11–13% protein, >250 s HFN and >76 kg/hl specific weight) with both spring and winter-wheat crops in Northern Britain [7,8]. In conventional farming systems, foliar mineral-N fertiliser applications later in the growing season are used to increase not only yield, but also protein content and other processing quality parameters [30]. However, water-soluble, readily plant-available mineral-N fertilizers are prohibited in organic farming systems and EU environmental regulations limit total annual pre-planting N-inputs with manure to 170 kg N/ha [8,9].

Achieving baking/milling quality is therefore a particular challenge in organic farming systems in Northern Britain. For example, when the effect of organic and conventional production protocols on performance of a modern short-straw winter-wheat variety (Malacca) was assessed in four contrasting growing seasons organic crops produced yields of ~5 t/ha and grain protein contents of ~10.5% while conventional crops produced yields of ~7 t/ha and protein contents of ~11.5% [7].

Results of this pilot study indicates that may enable wheat yields in organic farming systems to be increased to levels close to the average conventional wheat yield of ~7 t/ha achieved in Northern Britain, while at the same time achieve baking or milling grade quality. It is important to point out that this pilot trial was only carried out in one growing season and that both winter- and spring-wheat yields vary considerably between growing seasons in Northern Britain [7,8].

- changes to the management of clover leys (i.e., use of Rhizobium inocula, application of high C:N-ratio greenwaste compost)

- use of winter-wheat varieties developed for lower-input conventional (Greina) or organic (Pollux, Wenga) production systems in Switzerland or
- use of spring wheat varieties developed in both the UK and Europe

However, a recent factorial field experiment in the same region of the UK reported that under organic farming conditions a long-straw variety (Azsita) from the same organic farming focused Swiss breeding programme as Pollux and Wenga produced significantly higher protein levels and similar yields to a short-straw variety (Solstice) which was developed for the conventional farming sector in the UK in two contrasting growing seasons [9].

Results from the clover establishment and nodulation assessments suggest that the effect of *Rhizobium* inocula on winter-wheat yields and protein content were due to an increase in symbiotic N-fixation by the clover ley (especially in year 1) and higher levels of residual soil N being available to the subsequent wheat crop. This view is supported by (a) the numerically ~35% higher available N concentrations in October 2004 in plots where clover leys had been established with *Rhizobium* inoculate clover seed, (b) higher tiller number in both spring and winter-wheat and (c) the slightly higher leaf chlorophyll levels at GS55 in winter-wheat grown after *Rhizobium* inoculated clover leys. However, when spring wheat plots were assessed on the same date, crops grown after *Rhizobium* inoculated clover leys had lower chlorophyll levels than those grown after non-inoculated clover leys. This could have been due to spring wheat being at an earlier growth stage (GS37) or to differences in N-losses and/or N-mineralisation and availability pattern from clover leys incorporated in autumn (before planting of winter-wheat) or early spring (before planting of spring wheat), and should be further investigated in future trials.

The higher protein content in winter-wheat compared with spring-wheat and the higher yield of the variety Greina (but not the other two-winter-wheat varieties) resulting from greenwaste compost applications to clover leys are more difficult to explain and probably due to complex relationships between (1) NH_4^+ being released as a result of N-mineralisation after clover biomass was incorporated into soil in autumn, (2) increased N-assimilation by the soil biota in response to high C:N ratio (13:1) compost applications reducing NH_4^+ volatalization and NO_3^- leaching losses and thus increasing the plant availability N in soil and (3) differences in N-use efficiency between the three winter-wheat varieties. Such complex relationships may also explain the interactions between variety, *Rhizobium* seed inoculation and compost amendments observed for bread making quality parameters such as grain protein content, specific weight and hardness.

The finding that greenwaste compost applications slightly increased leaf chlorophyll levels and grain protein content in winter-wheat and did not affect leaf chlorophyll and protein levels in spring-wheat suggests that the high C:N ratio compost did not result in immobilisation/locking-up of N as has been previously reported for organic fertilisers, composts and plant residue inputs with a C:N-ratio of >20 [31–34].

Although clover assessments indicated that N-fixation in clover leys established with *Rhizobium*-inoculated seed was higher, grain protein concentration of winter-wheat grown after *Rhizobium*-inoculated clover leys were lower than those recorded in crops grown after leys established from non-inoculated seed. Since winter-wheat grown after *Rhizobium*-inoculated clover leys produced significantly higher vegetative biomass (tiller numbers) and grain, the lower protein contents could have been due to a "dilution effects" or differences in N-availability pattern (i.e., *Rhizobium* inoculation of preceding clover leys resulting in higher N-availability before and during tillering of in soils, but lower N-availablility at later growth stages of winter-wheat (e.g., during grain filling) when compared to N-availability pattern to wheat grown after non-inoculated clover leys.

It is unclear why clover management had no or only very small effects on yield and other performance parameters in spring-wheat, although (1) contrasting climatic conditions (temperature and rainfall) and associated differences in affecting N-mineralisation, N-losses and N-availability from clover leys incorporated in autumn and May and (2) contrasting

N-demand and uptake pattern of autumn and spring planted wheat crops may have been responsible, as previously described [35–37].

4.2. Nutritional Quality

Increasing N-supply by application of both (a) mineral N-fertilisers and (b) growing wheat crops after grass clover leys was previously shown to reduce concentrations of plant resistance-related phenolic compounds and other antioxidants in wheat and other crop plants [4,6,8,9,38]. In the study reported here, we assessed concentrations of Vitamin E in grain, since wholegrain cereals are an important source of Vitamin E and because there is limited information of the effect of cereal management practices on antioxidants other than phenolics [4,39,40].

Different to previous reports for phenolic antioxidants [6,8,9], we detected no significant effects of clover management and associated N-availability on Vitamin E concentrations in both winter- and spring-wheat when we assessed the main tocopherol and tocotrienol isomers found in wheat grain [40]. This is thought to be linked to the different functions and regulation of phenolic and tocopherols (=tocopherol and tocotrienol isomers) in plants. Specifically phenolic compounds (many of which were shown to have antimicrobial activity), which are part of the constitutive and inducible plant resistance response against attack by pathogens and pests [41,42] and their synthesis is known to be down-regulated in crop plants by high N-availability [6,43]. In contrast, the known roles of tocopherols include reduction of oxidative stress, maintainance membrane stability and intracellular signalling, and they are also thought to be involved in cyclic electron transport around photosystem II [44,45]. Similar to the results reported here increasing N-fertilization and availability was previously found not to affect or increase tocopherol concentrations in plants [46–49].

The finding that there are significant differences in tocopherol and tocotrienol concentrations and isomer profiles between wheat varieties is also consistent with results from previous studies. For example, Lampi et al. [39] compared tocopherol and tocotrienol concentrations in 175 genotypes of, primarily bread wheats, including 130 and 20 winter- and spring-wheat types. The average total content of tocopherols and tocotrienols was 49.4 μg/g of dm, with a range of 27.6−79.7 μg/g of dm, indicating a 2.9-fold variation among genotypes. Similarly, Hussain et al. [49] compared α-tocopherol levels in contrasting landraces, primitive, older and modern cultivars of wheat and reported significant differences between varieties grown under organic farming conditions, with the highest α-tocopherol levels found in long-straw landraces and the lowest in modern shorter straw cultivars.

5. Conclusions

Results indicate that improvements in clover-management practices and selections of wheat varieties suitable for organic farming systems have the potential to increase grain yield and/or allow farmers to reliably achieve baking or milling grade quality, provided wheat crops are grown immediately after clover leys. However, results from this pilot study should be confirmed in future field trials in sites with contrasting pedoclimatic conditions, before general recommendations are made.

The study also provides further evidence that longer-straw and in particular cultivars specifically bred for organic farming systems, may be more suitable for organic farming systems.

Similar to phenolic antioxidants, the study found that there is significant variation on Vitamin E content between varieties, which demonstrates the potential to breed/select varieties for high nutritional quality in the future. However, different to phenolics, our study found no evidence that Vitamin E concentrations are significantly affected by high N-availability

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092085/s1, Supplementary Figure S1. Location of Gilchester Farm in Northern Britain; Supplementary Table S1. Effect of soil depth at which soil samples were taken, *Rhizobium* inoculation of clover seed and greenwaste compost application on total nitrogen (N), nitrate (NO_3^-), ammonium (NH_4^+) and total plant available N (NO_3^- + NH_4^+) concentrations in soils in October 2005. Supplementary Table S2. Effect of soil depth, *Rhizobium* inoculation of clover seed and greenwaste compost application on total carbon (C), phosphorus (P) and potassium (K) concentrations in soils in October 2005.

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