Effects of agronomic management and climate on leaf phenolic profiles, disease severity and grain yield in organic and conventional wheat production systems

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Abstract

Agricultural intensification over the last 40 years has increased cereal yields, but there is very limited information on the effects of intensification practices (e.g. non-diverse rotations, mineral NPK fertiliser and pesticides) on crop health and quality. Results from the study reported here suggest that the use of mineral NPK fertilisers reduces phenolic acid and flavonoid concentrations in leaves, and increases the susceptibility of wheat to lodging and powdery mildew, when compared to composted FYM inputs. In contrast, the use of herbicides, fungicides and growth regulators reduces lodging and foliar disease severity, but had no effect on phenolic acid and flavonoid concentrations. The use of composted FYM inputs also resulted in a significant grain yield reduction and not substantially reduced the severity of opportunistic pathogens such as *Septoria*, which remain a major yield limiting factor unless fungicides are used and/or more *Septoria* resistant varieties become available.

Keywords: Wheat, Organic agriculture, Powdery mildew, *Septoria tritici*, phenolic profiles, Composted FYM, Mineral fertiliser, Disease control

Introduction

There is increasing concern over the dependence of agricultural food production on mineral fertiliser (especially nitrogen [N], phosphorus [P] and potassium [K]) and synthetic chemical pesticides, since these inputs are associated with significant negative environmental impacts and reduce the sustainability of crop production systems [1]. On the other hand, the introduction of herbicide and mineral NPK input-based minimum-till systems has reduced certain negative environmental impacts (e.g. wind and water erosion) and allowed commercially viable arable production in many semi-arid and arid regions (e.g. in the US and Australia) [2]. However, the long term sustainability of such systems has also been questioned, due to their reliance on non-renewable resources (e.g. P and K fertilisers) as well as the development of herbicide resistance [3]. The reliance of food production on mineral N fertilisers is of particular concern because their manufacture and usage is associated with high levels of fossil fuel/energy use and greenhouse gas emissions, in addition to increasing eutrophication of fresh water and marine ecosystems [1]. Concern has also been raised about the decline in nutritional value of the harvested products such as grains [4-7] as well as the rapid exploitation of global phosphorus deposits, which are predicted to be depleted within the next 100 years [8]. Without P fertiliser inputs yields in conventional farming systems could decrease to levels obtained prior to the widespread use of mineral fertilisers in the early 1900, unless alternative P-recycling based fertilisation methods are introduced[9]. The need for improved recycling of P and other mineral nutrients (e.g. via increased use of animal and green manures and urban organic wastes) into agricultural soils is already recognized in countries such as Australia, where the bulk of agricultural products (and the P contained within them) are exported and large volumes of P fertiliser have to be imported to sustain or improve the fertility of the naturally P-impoverished soils [10].

Organic farming is less dependent on mineral NPK fertiliser inputs, since it recycles NPK via animal manures, legume crops and organic waste-based fertilisers [11]. Organic farming standards prohibit the use of chemosynthetic pesticides, mineral N, water soluble P-

fertilisers such as superphosphate and potash (KCl), and restrict the use of raw phosphate and K_2SO_4 fertilisers. Instead, organic production relies on (i) diverse crop rotations, mechanical, physical and biological crop protection approaches/products and a limited range of plant extract (e.g. pyrethrum)- and mineral (e.g. S and Cu)-based crop protection products and (ii) recycling of mineral nutrients via green and animal manures and appropriately treated (e.g. composted or anaerobically digested) organic wastes to maintain soil fertility [12].

Recent systematic reviews suggest that organic crop production systems currently produce on average 30% lower yields when compared to intensive conventional crop production systems [13, 14]. However organic farming may provide a range of other benefits including: (i) higher soil carbon and organic matter levels, biological activity and biodiversity [15, 16], (ii) lower negative environmental impacts and carbon footprints [17], [18-20], (iii) reduced use of non-renewable and/or scarce natural resources (fossil fuel, P, K and other minerals used as fertilisers) [19] and (iv) improved nutritional quality of crops (e.g. higher antioxidant, and lower cadmium and pesticide residues) [21]. However, it should be noted that higher organic matter levels on organic farms may be, at least partially, due to the use of imported organic fertilisers (including manure from non-organic farms)[22].

Recent studies also indicate that the frequent use of organic fertiliser inputs (e.g. composted manure) may contribute to (i) increased soil biological activity, structural stability, erosion resistance [23], (ii) suppression of soil-borne diseases [24], (iii) induced resistance against certain foliar and vascular diseases [24, 25] and (iv) higher inherent soil fertility (yield per unit fertiliser input) [26, 27]. Organic fertiliser inputs therefore not only reduce the pressure/need for mineral NPK, but also pesticide or thermal crop protection treatments for foliar and soil-borne diseases[28].

It has also been suggested that the non-use of mineral fertilisers (and in particular N-fertilisers) in organic farming systems will result in higher concentrations of phenolic-based "resistance" compounds and increased resistance against lodging and foliar fungal diseases (in particular obligate pathogens such as powdery mildew and rusts), [29-31]. Yield losses in cereals due to fungal diseases have been estimated to be between 20% and 40% globally,

and in the USA rust and *Fusarium* head blight alone are estimated to result in economic losses of around $8 billion per year [32, 33].

Phenolic compounds are known to confer resistance to foliar diseases [34 29, 35-40]. Plant cells mainly contain phenolics in inactive bound “storage” forms. These inactive precursor compounds are rapidly converted into biologically active “free” forms by hydrolysing enzymes such as glycosidases, which are known to be produced by the plant in response to tissue damage or pathogen attack. Free phenolics have been shown to have a higher antimicrobial activity than bound forms [41].

Infection of cereals by fungal pathogens was also shown to result in an accumulation of hydroxycinnamic acid conjugated with polyamine derivatives, flavonoids, phenolglucosides and lignin [42]. A recent review by Balmer et al. [43] has summarised the role of these metabolites in the response to fungal pathogens in cereals. Most studies on the contribution of phenolics in crop resistance have focused on *Fusarium graminearum* in barley where resistance was closely linked to an activation of the phenylpropanoid, terpenoid, and fatty acid metabolic pathways [38]. The activation of specific phenolic pathways in wheat was also linked to resistance against Karnal bunt (*Neovossia indica*) [35] and spot blotch (*Bipolaris sorokiniana*) [36]. In contrast, there is less mechanistic information on the contribution of phenolic metabolism to powdery mildew and *Septoria* resistance in wheat. Phenolic compounds are also known to convey resistance to abiotic stress conditions such as drought , salinity [44], soil flooding , temperature stress [45], excessive UV irradiation and CO_2 levels [46].

Phenolics (which includes phenolic acids, flavonoids, stilbenes, coumarins, and tannins) are plant secondary metabolites with antioxidant activity that are known to affect the nutritional and sensory (colour and taste) quality of crop plants [47]. A range of studies have shown that both the composition and concentrations of phenolics, other antioxidants and/or total antioxidant activity in crops may be affected by genotype, environmental conditions and agronomic management parameters (especially fertiliser type and input level) [21, 48-52].

The objectives of this study were to (i) quantify the effect of and interactions between rotation, crop protection and fertility management practices used in organic and conventional cropping systems on leaf phenolic profiles, wheat disease severity and grain yields and (ii) identify associations between environmental and agronomic management practices on phenolic profiles and disease severity in wheat.

Materials and Methods

Site description

An existing long-term field experiment, the Nafferton Factorial Systems Comparison (NFSC) trial was used. The NFSC is a factorial field experiment with agronomic practices that differ between organic and conventional production systems (rotation design, fertilisation regimes and crop protection methods) as factors. It was established in 2001 on a field with a uniform sandy loam at Nafferton Experimental Farm (54:59:09 N; 1:43:56 W, Newcastle University) to study the effects of organic, low agrochemical (pesticide or mineral NPK) input and conventional production protocols on crop productivity, sustainability, environmental impacts, food quality and safety.

Field experimental design

Nafferton Factorial Systems Comparison (NFSC) trial is a split-split-split plot design with 4 replicate blocks and (1) pre-crop as the main plot factor, (2) crop protection as the sub-plot factor and fertility management as the sub-sub-plot factor as described in detail in the supporting information and by Eyre et al. [53]. This design allows the experiment to be analysed as a 2 x 2 x 2 factorial experiment with preceding (pre)-crop (a diverse rotation prescribed for organic systems vs non-diverse cereal dominated rotation typical for conventional systems), crop protection (with and without use of synthetic pesticides, fungicides and herbicides), and fertility management (composted manure vs NPK-fertiliser inputs typically used in organic and conventional farming systems respectively) as factors. The four agronomic protocols compared in both the diverse and non-diverse rotational background (=main plots) are therefore: (1) fertilisation with composted cattle manure and organic crop protection (organic management); (2) fertilisation with composted cattle manure and conventional crop protection; (3) Mineral NPK fertilisation and organic crop protection; (4) Mineral NPK fertilisation and conventional crop protection (conventional management).

The NFSC-trial includes 4 “replicate” experiments (with very similar designs), which started the rotational sequence with a 1st wheat crop in different years between 2003 and 2006 resulting each crop in the rotational sequence being grown twice over a 4 year period (see Table S1). This is designed to rapidly generate crop performance data in contrasting growing seasons/ climatic background conditions.

Agronomic protocols used

Winter wheat (variety Malacca, a UK bread-making variety) was sown in late October (in 2004, 2006, 2007 and 2008) by using a commercial drill (3m Lely combination drill; Lely UK Ltd, St Neots, UK). Seed used in conventional crop protection plots was supplied by Horizon Seeds Ltd. and was produced using standard commercial seed production protocols which included pesticide and fungicide seed treatments. Seed used in organic crop protection plots was also supplied by Horizon Seeds Ltd., but produced according to organic seed production standards (Soil Association, Bristol, UK) and were untreated (no fungicide or insecticide seed dressings). Details of the fertilisation and crop protection protocols (including products used, application timings and rates) and the climatic conditions in the 4 different growing seasons are provided in the supporting information Tables S2 and S3).

Crops from all plots/treatment combinations were harvested in late August in 2005, 2007, 2008 and 2009 using a plot combine harvester (Claas Dominator 38; Claas U.K Ltd, Bury St Edmunds, UK) and grain samples were dried (hot air drying using an electric motor fed through a 3m x 1.5m x 0.70m wooden box with a meshed surface for grain sacks to rest on) and cleaned (Lainchbury HC1/ 7W grain cleaner, Blair Engineering, Blairgowrie, UK) immediately after harvest. Further background information/ data from the NFSC trial relating to metal contents in wheat [11], life-cycle analysis of greenhouse gas emissions [54], beneficial invertebrates distribution [27], weed cover [53] and diversity of free-living N fixing bacteria [55] have been published previously.

Lodging and disease assessments and wheat flag leaf sampling

Powdery mildew (*Blumeria. graminis f. sp tritici*) and Septoria leaf Blotch (*Septoria tritici)* disease severity (% infected leaf area) were assessed weekly after the first disease symptoms were detected as described in Bilsborrow et al. [56]. Leaning/lodging severity was also assessed (before harvest) and the % area of a plot showing leaning/lodging recorded. Leaning/ lodging was defined as cereal tillers/stems being bent over at an angle of ≥45° or laying on the ground. The severity of stem based diseases, which are a contributing factor to leaning/lodging, was not recorded.

Wheat flag leaves were sampled from each plot at late booting (Growth Stage 50, GS50) [57]. In 2009 samples were also taken at GS62 and GS71. Approximately 100 – 200 flag leaves from each plot were collected and immediately frozen, lyophilised and milled as described in Tetard Jones et al. [58]. At GS50 plants in all plots were visually free of foliar disease on the youngest 4 leaves, and there were no soil-borne or stem-base disease symptoms.

Analysis of leaf mineral N, P and K concentrations

Flag leaves (fine powder) were analysed for total N by Dumas combustion (LECO TruSpec Automated C/N Analyzer, LECO Corporation, USA). Other nutrients (P, K) were determined following acid digestion (H_2O_2, HNO_3) of leaves in a closed-vessel microwave reaction system (MarsExpress; CEM Corp., Matthews, NC, USA) and analysed with an inductively coupled argon plasma optical emission spectrometer (ICP-OES) equipped with a CCD detector (Vista-Pro Axial; Varian Pty Ltd, Mulgrave, Australia) as described previously [11].

Analysis of leaf phenolic acid and flavonoid concentrations

A standard method was used for the extraction of phenolics from leaves as described by Bennett et al. [59]. Extracts were analysed by HPLC on a Shimadzu Prominence HPLC system equipped with an LC-20AD pump, SIL-20AC autosampler, and SPD-M20A photodiode array detector (Shimadzu Corp., Kyoto, Japan). Data collection and integration were performed using Shimadzu LC solution software. Phenolic acids and flavonoids were separated on a

reverse-phase Thermo Scientific Hypersil C18 column (250 × 4.6 mm, 5 µm). The column was heated at 25˚C while the sample tray temperature was set to 4°C. Eluent A was 0.1% (v/v) aqueous trifluoroacetic acid (TFA), while eluent B was 0.1% acetonitrile (CH_3CN), and the solvent gradient was programmed as follows: The elution profile for the best method was 0 mins 0% CH_3CN; 0-5 mins 0% CH_3CN; 5-50 mins 35% CH_3CN; 50-55 mins 45% CH_3CN; 55-60 mins 0% CH_3CN; 60-65 mins 0% CH_3CN. The flow rate of the mobile phase was 1.0 mL/m, and the injection volume was 20 µL. Scanning was performed from 200nm to 600 nm, and phenolic acids were identified by comparing retention times and UV-VIS spectra with those of pure standards. Concentrations, expressed in µ/g of dry matter, were calculated at 227, 270, or 320nm using calibration curves of phenolic acid standards and flavonoids that underwent the same extraction procedure.

Statistical analysis

The effects of year, crop protection and fertility management on wheat grain yield, disease severity and flag leaf phenolic concentrations were assessed using ANOVA derived from linear mixed-effects model [60]. The hierarchical nature of the split-split-plot design was reflected in the random error structures that were specified as block/year/crop protection. Where analysis at a given level of a factor was carried out, that factor was removed from the random error term. Another model with previous crop, crop protection and fertility management as fixed effects was used for data analysis from years in which wheat crops were grown following more than one species of pre-crop (2007, 2008). The hierarchical nature of the split-split plot design was reflected in the random error structures that were specified as block/pre-crop/crop protection. In 2005 and 2009 when wheat was grown after only one previous crop, the model only included crop protection and fertility management as fixed effects. This reduced model was also used when previous crop did not have a significant effect. The normality of the residuals of all models was tested using QQ-plots. Differences between the four crop management strategies used (FM x CP interaction means) and interactions between management strategies and pre-crop were tested using

Tukey contrasts of the general linear hypothesis testing (glht) function of the multcomp package in R. A linear mixed effects model was used, containing a treatment main effect, with four levels, with the random error term specified as described above. Standard error (SE) of mean was used in order to describe how precise the mean of the sample is compared with the true mean of the population. Both means and SE were generated by using the ‘’t apply’’ function in R.

The relationships between leaf phenolics, disease severity, environmental and agronomic factors, were investigated using redundancy analysis (RDA). In all cases the RDAs were carried out using the CANOCO 5 package [61]. Automatic forward selection of the environmental, agronomic and phenolic factors within the RDAs was used and their significance in explaining additional variance calculated using Monte Carlo permutation tests. Relative humidity and soil temperature were not used as drivers in RDAs since they were auto-correlated with rainfall and air temperature, respectively. Pearson parametric correlation test, Spearman and Kendall rank-based correlation analysis were performed using the correlation testing (cor.test) function in R. Correlation matrixes were visualized with correlograms using corrplot package in R.

Results and discussion

Phenolic profiles

Phenolic compounds are plant secondary metabolites derived from the phenylpropanoid pathway with flavonoids and phenolic acids being the major groups found in cereals[43, 62, 63]. Phenolic acids and flavonoids account for a substantial proportion of total antioxidant activity in cereal leaves and grains, and dietary intake of plant (poly) phenolics have been linked to a range of positive health impacts [64, 65].

In the current study ANOVA results show that phenolic acid and flavonoid concentrations in flag leaves was affected mainly by fertilisation (composted manure vs mineral NPK fertiliser), and to a lesser extent rotational position/ pre-crop, but not crop protection regimes (with and without the use of synthetic chemical pesticides) (Table 1; Table S7). The use of mineral NPK fertiliser resulted in lower phenolic concentrations (Table 1), and in one season (2008) the use of grass-clover pre-crop resulted in lower phenolic acid concentrations in flag leaves (Table S7). Very similar responses were found for most individual phenolic acids and flavonoids (Tables S9-S12). In field experiments composted FYM was applied at similar total N-input levels to mineral NPK fertilisers (170 vs 180-210 kg N ha^{-1}), but it is well known that N (but also K) release/ availability from manure is substantially lower and slower than from mineral fertilisers [66]. This was confirmed by flag leaf analysis which showed 25% higher N, 10% higher K, but similar P concentrations in mineral NPK compared to composted FYM fertilised crops (Tables S16-S22). These findings are consistent with previous studies which showed that high mineral N fertiliser inputs reduce levels of phenolic compounds in plant tissues[30, 67, 68].

In 2009 phenolic profiles in flag leaves were assessed at 3 different growth stages (GS 50, 62 and 71) to **(a)** determine whether phenolic concentrations in flag leaves change over time and **(b)** identify potential associations between diseases severity and phenolic concentrations/ profiles (Table 2). When growth stage was included as a factor in the

ANOVA there were significant main effects for GS and fertilisation, but not for crop protection. Mineral fertiliser resulted in significantly lower total phenolic acid and flavonoid concentrations and the effect of fertilisation on leaf phenolic profiles was similar at all 3 growth stages (no significant interactions between growth stage and agronomic factors [fertilisation and crop protection] were detected) (Table 2). Between GS50 and GS62 total phenolic acid and flavonoid concentrations increased, but then decreased again between GS62 and GS71 to levels lower (phenolic acids) or similar (flavonoids) to those found at GS50 (Table 2).

These results suggest that the greater availability of N, (and possibly K) associated with the use of mineral NPK fertilisers reduced the biosynthesis of total phenolic compounds in wheat leaves between GS50 (booting) and GS71 (early milking) (Tables S23-S25). This conclusion is also supported by correlation analyses which showed weak negative associations between leaf N and leaf phenolic/flavonoid compounds at GS50 and GS62 and a strong negative association at GS72 (Figure S2b-d).

Associations between agronomic and environmental factors and phenolic concentrations in flag leaves at GS50

The conclusion that N-availability is a major driver for phenolic concentrations in wheat leaves is also supported by results from the redundancy analysis (RDA), which identified fertilisation regime, N supply (estimated based on flag leaf N concentrations) and the use of grass/clover pre-crops as negative drivers for phenolic concentrations.

In the bi-plot shown in Figure 1a, associations between agronomic (organic versus conventional fertilisation and crop protection regimes), macronutrient supply (estimated based on flag leaf NPK concentrations) and climatic (radiation, precipitation and air temperature) factors, and phenolic compounds in flag leaves were observed. Mean weather data from the 4 weeks prior to flag leaf sampling (GS50) were used as drivers, since this was assumed to be the most important period for regulation of phenolic compound concentrations. Very similar results were obtained when weather data from 14 weeks prior to

315 flag leaf sampling (GS50) were used in the RDA (Figure S1). Most variation (60%) is
316 explained by axis 1 and a further 18% by axis 2.
317 Concentrations of total chlorogenic acid derivatives and all individual phenolic
318 compounds (except for neo-chlorogenic acid) were negatively associated (along the
319 negative axes 1 and 2) with mineral NPK fertilisation, N (and to a lesser extent K) supply,
320 and use of grass clover leys, while crop protection explained virtually none of the additional
321 variation (Figure 1a). In contrast, flag leaf flavonoid and phenolic acid concentrations were
322 positively associated with organic fertilisation, radiation and temperature along the positive
323 axis 1. Total flavonoid concentrations were more closely positively associated with
324 temperature along the positive axis 1 and negative axis 2, while concentrations of total
325 chlorogenic acid derivatives were more closely associated with organic fertilisation, winter-
326 wheat as a pre-crop and to a lesser extent radiation along the positive axes 1 and 2 (Figure
327 1a).
328 The finding that the only major pre-crop effect detected was a reduced concentration
329 of both phenolic acid and flavonoid concentrations in wheat following grass/clover (when
330 compared to potato and winter-wheat pre-crops) (Table S7) is also consistent with a
331 regulation of phenolic expression through N-supply /availability pattern. Grass/clover leys
332 are known to result in substantially higher N-availability than wheat and potato pre-crops,
333 especially in organic production systems[56, 66, 69]. However, it should be noted that differences
334 in phenolic compounds may have been due to differences in **(i)** the total supply/availability N
335 (and possibly K), **(ii)** nutrient release patterns during the growing period and/or **(iii)** the ratio
336 of N:P:K available to crops. A major focus of future studies should therefore focus on
337 identifying the effects of these 3 parameters on the regulation of phenolic expression in
338 wheat cultivars with contrasting foliar disease resistance. This could potentially identify
339 fertilisation strategies which increase productivity without reducing phenolic acid and
340 flavonoid concentrations in both wheat leaves (which may increase resistance against
341 biothropic pathogens), and cereal grains (which may increase grain nutritional value).
342 Previous studies showed that organic fertilisation regimes also increased phenolic

concentrations in wheat grain suggesting that leaf and grain phenolic concentrations are correlated[50-52]. Also, for consumers of "wheatgrass"-based smoothies (which are increasing in popularity and often advertised as "superfoods;[70] the higher phenolic concentrations in wheat leaves may be of direct nutritional relevance.

Several previous studies concluded that the higher antioxidant/ (poly)phenolic concentrations found in organic crops is due to the non-use of pesticides resulting in greater disease severity and induction of plant resistance mechanisms that lead to antioxidant/ (poly)phenolic synthesis [71, 72]. However results from this study do not support this hypothesis with no significant differences in phenolic concentration being observed between crops grown under conventional and organic crop protection.

As in previous studies high solar radiation and low relative humidity were positively associated with antioxidant/ phenolic concentrations in crops [73]. However, the finding that the strength of associations between phenolic concentrations and fertilisation was similar to that found for relative humidity/ radiation has not been reported previously. While it is extremely difficult to change/ optimise humidity and radiation levels in field crop production systems, the results reported here suggest that it is possible to optimise phenolic concentrations in wheat by switching to organic fertilisers, or by reducing or optimising the use of mineral N-fertilisers (e.g. split applications). This should be further investigated in future studies.

Foliar disease severity

Disease severity and lodging differed substantially between seasons (especially powdery mildew severity) and was lower when conventional, pesticide-based crop protection regimes (with plant growth regulators) were used (Table 3). This and the finding of strong positive associations of both powdery mildew and *Septoria* severity with radiation and relative humidity and negative association with temperature (Figure 1b) is consistent with results a previous study into effects of climatic conditions and fungicides on *Septoria* and powdery mildew in winter wheat crops grown in the North East of England [56].

When the effect of agronomic factors on foliar disease severity was assessed, significant main effects of both crop protection and fertilisation were detected, except for *Septoria* severity on the flag leaf, for which no significant main effect of fertilisation was detected (Table 3). There were also significant interactions between crop protection and fertilisation for *Septoria*, powdery mildew and lodging severity (Figure 2b-d).

Use of mineral NPK-fertiliser resulted in significantly lower *Septoria* (on L2 leaf) severity, but significantly higher powdery mildew severity and lodging than use of composted manure (Table 3). The finding of a higher incidence of lodging and powdery mildew in crops fertilised with mineral NPK rather than composted manure agrees with previous studies into the effect of different fertiliser types and N input levels on the severity of lodging, powdery mildew and other biothrophic diseases (e.g. rusts, *Fusarium*) in cereals [74-76] and other crops [77]. Future studies should investigate whether this is resulted from differences in total N-availability or from differences in N-availability pattern over the growing season.

There were significant, but contrasting interactions between fertilisation and crop protection for *Septoria,* powdery mildew and lodging (Figure 2b-d). *Septoria* disease levels were substantially reduced by pesticide-based conventional crop protection and the lowest disease severity was found in crops treated with both mineral NPK fertilisers and pesticides (Figure 2b). In contrast, powdery mildew and lodging were virtually absent in crops fertilised with composted manure. Lodging was observed in 3 of the 4 years (2005, 2007 and 2009) and only in mineral fertilised plots (Figure 2d). Statistical analysis of the effect of pesticide/growth regulator use in mineral fertilised plots on lodging was only possible in 2007, the year with the highest lodging severity, and found to be significantly lower in crops under conventional crop protection (Figure 2b). Similarly, the use of pesticides in mineral fertilised crops resulted in a significant reduction of powdery mildew severity (Figure 2c). These results are consistent with previous studies reporting that high mineral N-inputs/ availability increases the severity of lodging, powdery mildew and other bio trophic diseases (e.g. rusts, *Fusarium*) in cereals [74-76] and other crops [77].

In 2008 wheat grown after wheat as a pre-crop i.e. a 2nd wheat resulted in higher *Septoria* disease severity on flag leaves than pre-crops potato and grass clover (Table S26). However, there was an interaction between pre-crop and crop protection (Table S26) and significant differences between pre-crops were only found in crops under organic crop protection, while *Septoria* severity was very low and not significantly different between pre-crops under conventional crop protection (Table S27). This indicates that maintaining the use of diverse rotations (e.g. not growing wheat after wheat) is more important for organic farming systems, which do not allow the use of synthetic chemical pesticides.

These results are broadly consistent with results from field trials in which the performance of new wheat varieties was compared in organic and conventional agronomic backgrounds [78]. They also reported that *Septoria* severity was similar under the different management systems while the severity of biotrophic diseases such as powdery mildew and rusts was substantially lower in the organic production system.

Association between phenolic concentrations and disease severity/lodging

Many phenolic compounds have been shown to have anti-fungal activity and both phenolic acids and flavonoids have been linked to resistance against a range of cereal pathogens including powdery mildew [30], rusts [79], and *Fusarium* [29, 62]. A recent study also reported associations between lower phenolic concentrations in strawberry leaves (resulting from high mineral N-fertilisation) and higher pest severity (populations of two spotted spider mite) in strawberries [68].

However, to our knowledge there is currently no strong evidence for phenolic acids and flavonoids contributing to resistance against **(i)** eyespot and other stem based diseases contributing to lodging and **(ii)** *Septoria nodorum* blotch (SNB) and *Septoria tritici* blotch (STB), the economically most important wheat diseases in Europe [80]. RDA and correlation analysis results from this study showed strong negative associations between phenolic compounds and both powdery mildew and *Septoria* disease severity (Figure 3; Figure S2a-

d). This may indicate that high phenolic concentrations (and associated antifungal activities) are important in the resistance response to both powdery mildew[35, 79, 81] and *Septoria.*

Grain yield

Grain yield data used in this study were from winter wheat crops grown in the harvest seasons 2005, 2007, 2008 and 2009 (Table 3), seasons in which flag leaf phenolic profiles were also analysed. Grain yield data from the 2004, 2005, 2007, and 2008 seasons of the NFSC-trial has already been reported [56]. Grain yields were substantially (45%) lower in the organic compared to the conventional system, with less efficient crop protection and fertilisation each accounting for approximately half of the yield gap between the different production systems. This is larger than the on average 20-30% yield gap between organic and conventional cereals reported in the most recent meta-analyses of published comparative crop yield data [13]. However, while yields in conventionally managed plots (8 t ha^{-1}) are consistent with UK commercial yields the average yields on organic plots (4.4 t ha^{-1}) were lower than yields recorded in recent variety trials on commercial organic farms in the UK [82, 83].

This is thought to be at least partially due the fact that composted FYM inputs in experiments were at the level that could be sustained by the farm's dairy herd (170 kg N ha^{1}), while mineral fertiliser inputs were made at levels recommended for achieving optimum economic returns in conventional systems (180-210 kg N ha^{-1}). This, and results of the most recent meta-analyses comparing organic and conventional yields [13, 84] suggest that there is scope for substantially increasing yields in organic systems via both breeding (e.g. for nutrient use efficiency from organic fertilisers and improved *Septoria* resistance), improved fertilisation (e.g. higher organic fertiliser inputs, development of "precision" organic fertilisers) and better crop protection methods (e.g. development of crop protection products that are compatible with organic farming standards). In contrast, the finding that average winter wheat yields on commercial farms in the UK and other European countries have plateaued over the last 15 years [85] suggests that it may be more difficult to further increase

conventional wheat yields. It should also pointed out, that yields on the organic plots in the NFSC trial (which received no mineral N, P and K fertiliser inputs between 2001 and 2009) were higher than average yields of winter wheat in conventional production in the USA, Canada and Australia (4.2 t ha^{-1}, 4.0 and 2.0 t ha^{-1} respectively) [86, 87].

Overall the results of the study also indicate that nutrient-recycling focused agricultural production systems such as organic farming, may allow high grain yield levels to be maintained without mineral NPK fertiliser inputs, if manure, sewage and other organic waste based organic fertilisers are applied at total input levels permitted under current EU environmental legislation. In some countries, including Australia the use of animal and green manures green waste and bio solids is increasingly popular among non-organic farmers [10] and availability of manure and organic waste-based fertilisers may become a major bottleneck for the expansion of organic production. Furthermore, the development of "precision" organic fertilisers and fertilisation systems that increase the supply of N (and other nutrients) and productivity without increasing lodging or foliar disease severity are thought to be agronomic innovations that would improve the sustainability in both the organic and conventional arable sectors[88].

Furthermore, breeding for *Septoria* resistance and nutrient use efficiency from organic fertiliser inputs should be the most important future objectives for the organic sector, while the conventional sector requires breeding/selection for a wider range of resistance traits including (i) further improved lodging resistance and (ii) resistance against a range of bio trophic diseases (e.g. rusts, powdery mildew and *Fusarium*) and the necrotrophic pathogen *Septoria*. However, there is currently limited published information on the interactions between environment x agronomy x variety which are essential to identify varieties as well as informing breeding strategies to develop new genotypes with suitable balances of robustness, yield and quality traits for high and low input cereal production systems.

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Supporting Information description

The supporting information is organised into four sections: **(1)** Information about the sequence of crops, agronomic management as well as climatic conditions for the four seasons of the study that took place at the Nafferton Factorial Systems Comparison experiments. **(2)** Information about the effects of treatments on individual and total chlorogenic, hydroxicinamic acids and flavonoid compounds as well as macro and micro nutrient content in wheat flag leaves. **(3)** Information about the effects of pre-crop on grain yield and *Septoria* disease severity. **(4)** Extra multivariate analysis that is showing the relationship between weather conditions (in the 14-15 week period prior to leaf samples being taken) and phenolic compounds and correlation analysis that showing positive and negative correlations between chlorogenic, hydroxicinamic acids, flavonoids and other measured parameters.

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Tables and Figures captions

Table 1 Main effect means ± SE and *P*-values for the effects of harvest year, crop protection and fertility management on concentrations of total Chlorogenic acid, hydroxcinnamic acid, phenolic acid and total flavonoid in wheat leaves

Table 2 Main effect means ± SE and *P*-values for the effects of growth stage, crop protection and fertility management on concentrations of phenolic compounds in wheat leaves in the 2009 harvest season

Table 3 Main effect means ± SE and *P*-values for the effects of harvest year, crop protection and fertility management on grain yield, stem lodging, *Septoria* and powdery mildew disease severity (AUDPC) on the flag, L2 and L3 leaves

Figure 1 Bi-plot derived from redundancy analysis showing the relationship between weather conditions (in the 4 week period prior to leaf samples being taken), agronomic management and **(a)** phenolic compounds in wheat leaves and **(b)** disease severity in wheat leaves

Figure 2 a-d Interaction means ± SE for the effects of crop protection and fertility management on grain yield, stem lodging, *Septoria* and powdery mildew disease severity (AUDPC)

Figure 3 Bi-plot derived from redundancy analysis showing the relationship between phenolic compounds and disease severity conditions and phenolic drivers with disease severity responses in wheat leave

Tables

Table 1 Main effect means ± SE and *P*-values for the effects of harvest year, crop protection and fertility management on concentrations of total Chlorogenic acid, hydroxcinnamic acid, phenolic acid and total flavonoid in wheat leaves

	Total Chlorogenic acid	**Total Hydrox-cinnamic acid**	**Total phenolic acid**	**Total flavonoids**
	mg g^{-1} DW	mg g^{-1} DW	mg g^{-1} DW	mg g^{-1} DW
Harvest year				
2005 (n=16)	7.3 ±0.6c	*	*	6.2 ±0.2b
2007 (n=48)	9.1 ±0.4b	*	*	6.3 ±0.2b
2008 (n=64)	9.8 ±0.3b	7.82±0.407	17.93±0.645	6.8 ±0.4b
2009 (n=16)	14.9 ±1.1a	3.5±0.206	18.44±1.264	16.1 ±0.9a
Crop protection				
Organic (n=72)	10.4 ±0.6	6.76±0.597	18.37±0.878	7.6±0.4
Conventional (n=72)	9.8 ±0.5	6.71±0.506	17.75±0.748	7.5±0.4
Fertilisation				
Organic (n=72)	12.8 ±0.6	7.18±0.57	21.19±0.645	8.5±0.5
Conventional (n=72)	7.4 ±0.2	6.29±0.53	14.92±0.534	6.6±0.3
ANOVA				
Main effects				
Harvest year (HY)	**<0.0001**	**0.0382**	ns	**0.0023**
Crop protection (CP)	ns	ns	ns	ns
Fertilisation (FE)	<**0.0001**	**0.01**	**<.0001**	**<0.0001**
Interactions				
HY x CP	ns	ns	ns	ns
HY x FM	**<0.0001**	ns	**0.0016**	**<0.0001**
CP x FM	ns	ns	ns	ns
HY x CP x FM	ns	ns	ns	ns

Means labelled with the same letter within the same column are not significant different (Tukey's honestly significant difference test, $P < 0.05$). * not determined in 2005 and 2007

Table 2 Main effect means ± SE and *P*-values for the effects of growth stage, crop protection and fertility management on concentrations of phenolic compounds in wheat leaves in the 2009 harvest season

	Total chlorogenic acid	**Total hydroxcinnamic acid**	**Total phenolic acid**	**Total Flavonoids**
	mg g^{-1} DW	mg g^{-1} DW	mg g^{-1} DW	mg g^{-1} DW
Growth Stage				
GS 50 (n=16)	14.9±1.1b	3.5±0.2a	18.4±1.3a	16.09±0.9b
GS 62 (n=16)	18.8±1.8a	2.6±0.2b	21.3±1.9a	24.14±1.4a
GS 71 (n=16)	10.9±1c	1.7±0.2c	12.7±1.2b	14.5±0.7b
Crop protection				
Organic (n=24)	14.9±1.3	2.58±0.2	17.5±1.5	18 ±1.2
Conventional (n=24)	14.9±1.2	2.63±0.2	17.5±1.4	18.5±1.3
Fertilisation				
Organic (n=24)	18.03±1.3	2.9±0.3	20.9±1.5	20.1±1.2
Conventional (n=24)	11.8±0.7	2.3±0.2	14±0.8	16.3±1.2
ANOVA				
Main effects				
Growth stage (GS)	**0.0063**	**0.0058**	**0.0066**	**0.0009**
Crop Protection (CP)	ns	ns	ns	ns
Fertilisation (FM)	**0.0001**	**0.0211**	**0.0001**	**0.0034**
Interactions				
GS x CP	ns	ns	ns	ns
GS x FM	*T*	*T*	*T*	ns
CP x FM	ns	ns	ns	ns
GS x CP x FM	ns	ns	ns	ns

Means labelled with the same letter within the same column are not significant different (Tukey's honestly significant difference test, $P < 0.05$); *T*: *P* value >0.05 <0.1

Table 3 Main effect means ± SE and *P*-values for the effects of harvest year, crop protection and fertility management on Grain yield, and lodging

	Yield	**Lodging**[#]	**Septoria severity** (AUDPC)		**Powdery mildew severity**[##] AUDPC	
	t ha^{-1} DW	%	**Flag leaf**	**L2-leaf**	**Flag leaf**	**L2-leaf**
Harvest year						
2005 (n=16)	6.3±0.4	-	297 ±30a	780 ±122a	149 ±43a	245 ± 71a
2007 (n=48)	5.7±0.2	11.6±2.5	151 ±14b	267 ±16b	0.4 ±0.2b	2.4 ±0.9b
2008 (n=64)	5.9±0.2	4.6±1.4	59 ±10c	133 ±19c	0.0 ±0.0b	0.1 ±0.1b
2009 (n=16)	6.0±0.5	-	93 ±15bc	36 ± 8c	0.0 ±0.0b	0.2 ±0.1b
Crop protection						
Organic (n=72)	5.0±0.1	10.5±2.4	176 ±14	327 ±38	25 ±12	43 ±20
Conventional (n=72)	6.7±0.2	4.6±1.2	57 ±10	144 ±33	13 ±6	19 ±10
Fertilisation						
Organic (n=72)	4.9±0.1	0.7±0.7	111 ±16	271 ±48	0.8 ±0.5	2.4 ±2
Conventional (n=72)	6.8±0.2	14.4±2.3	122 ±13	199 ±21	37 ±13	60 ±22
ANOVA						
Main effects						
Harvest year (HY)	ns	ns	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Crop protection (CP)	**<0.0001**	ns	**<0.0001**	**<0.0001**	**0.0217**	**0.0010**
Fertilisation (FM)	**<0.0001**	**<0.0001**	ns	**<0.0001**	**<0.0001**	**<0.0001**
Interactions						
HY x CP	**0.0008**	-	ns	**0.0054**	*T*	**0.0235**
HY x FM	**<0.000**	-	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
CP x FM	**<0.0001**[1]	**0.0401**[1]	**<0.0001**[1]	**<0.0001**[1]	**0.0026**[1]	**<0.0001**[1]
HY x CP x FM	*T*	-	**0.0042**	*T*	**0.0062**	**0.0341**

Means labelled with the same letter within the same column are not significant different (Tukey's honestly significant difference test, $P < 0.05$)
[1] see Figure 2 (a-d) for interaction means ± SE
[#] Lodging was only occurred in 2007 and 2008 seasons; [##] AUDPC: days × % severity; *T*: *P* value >0.05 <0.1

Figure graphics

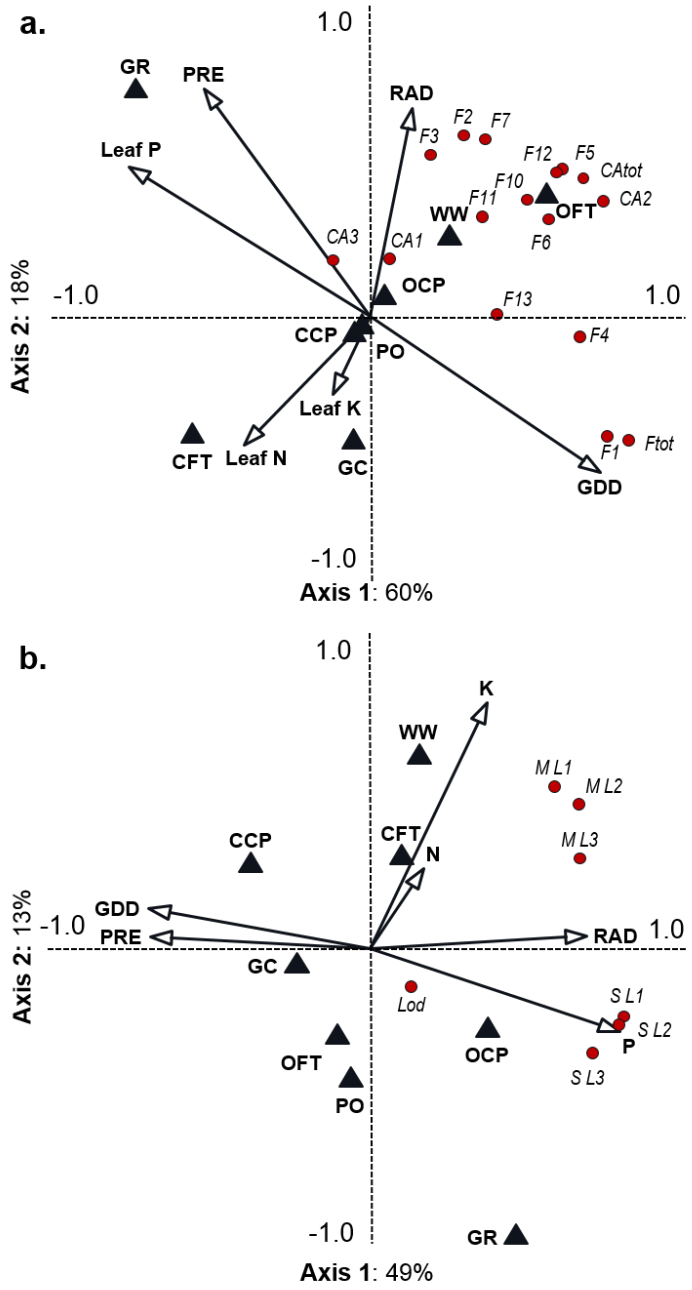

Figure 1 Bi-plots derived from redundancy analysis (RDA) showing the relationship between: **(a)** weather conditions, flag leaf NPK content, agronomic management drivers and Phenolic compounds in wheat flag leaves at GS50; **(b)** Weather conditions, flag leaf NPK content (GS50), agronomic management drivers and leaf disease severity (AUDPC)

CA1, neo-chlorogenic acid; CA2 chlorogenic acid; CA3, methyl-chlorogenic acid.; F1: isoorientin 6''-O-Xyloside; F2: mix of luteolin 6-C-galactoside, 8-C-glucoside and Luteolin 6, 8 Di-C-Glucoside; F3: apigenin 6-C-galactoside, 8-C-glucoside; F4: mix of uncharacterised apigenin and luteolin glycosides; F5: isoorientin; F6: isoorientin 2''-O-rhamnoside; F7: 3-C-glucoside, 3,4,2',4',6' Pentahydroxychalcone; F8: Isovitexin; F9: Isoscoparin; F10-12: uncharacterised flavonoid peaks; F13: tricin; S L1 = Septoria on flag-leaf; S L2 = Septoria on 2nd leaf; S L3= Septoria on 3rd leaf; M L1 = mildew on flag-leaf; M L2 = mildew on 2nd leaf; M L3 = mildew on 3rd leaf; Lod: stem lodging; CCP: conventional crop protection; OCP: organic crop protection; CF: conventional fertilisation; OF: organic fertilisation; PO: potato; GC: grass clover; GR: grass; WW: winter wheat; GDD: good degree days; PRE: precipitation; RAD: radiation. See tables S31 for Monte Carlo permutation test F and *P* values.

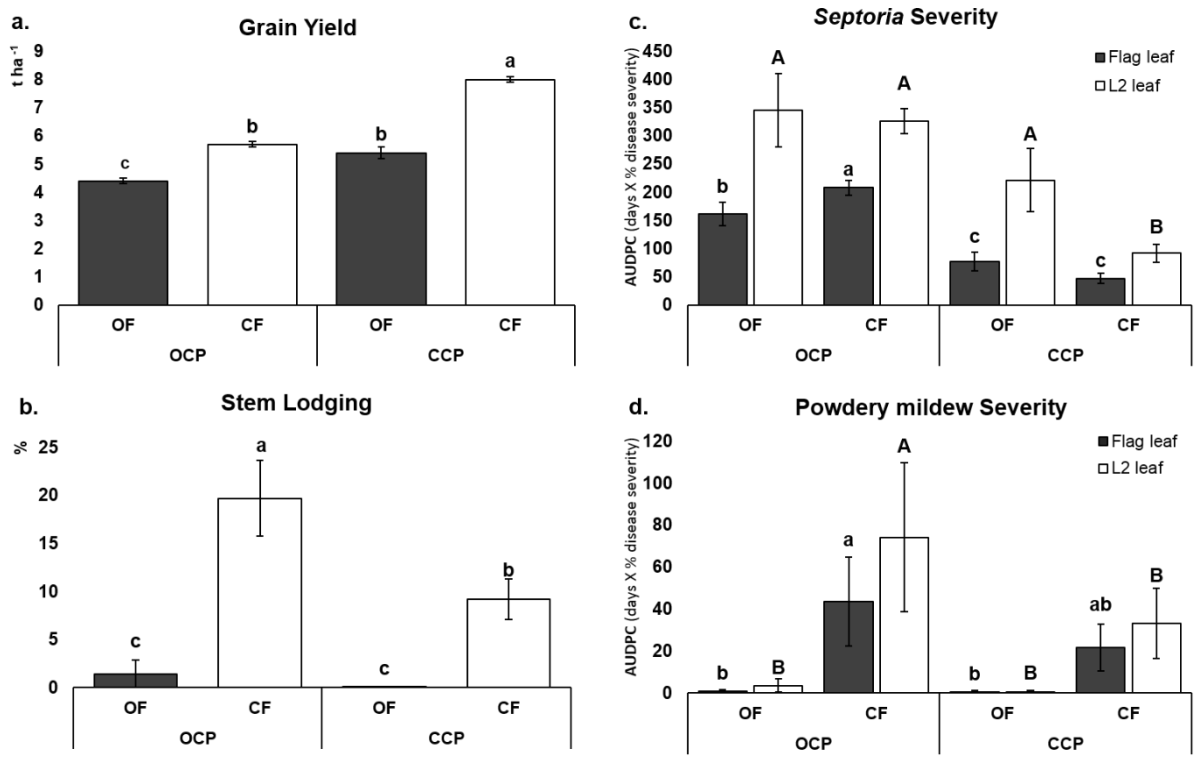

Figure 2 Interaction means ± SE for the effects of crop protection (OCP: Organic Crop Protection; CCP: Conventional Crop Protection) and fertility management (OF: Organic Fertilisation; CF: Conventional Fertilisation) on **(a)** grain yield, and **(b)** stem lodging at 2007 and 2008 **(c)** *Septoria* and **(d)** Powdery mildew disease severity (AUDPC day's × % severity)

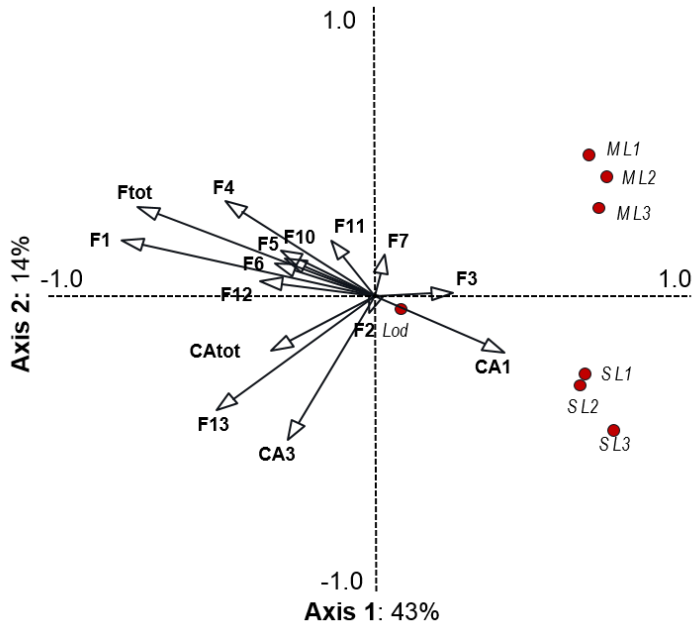

Figure 3 Bi-plot derived from redundancy analysis (RDA) showing the relationship between phenolic compounds (GS50) and disease severity (AUDPC) in wheat leaves. Eigenvalues were 43% and 14% for axes 1 and 2 respectively

CA1, neo-chlorogenic acid; CA2 chlorogenic acid; CA3, methyl-chlorogenic acid.; F1: isoorientin 6''-O-Xyloside; F2: mix of luteolin 6-C-galactoside, 8-C-glucoside and Luteolin 6, 8 Di-C-Glucoside; F3: apigenin 6-C-galactoside, 8-C-glucoside; F4: mix of uncharacterised apigenin and luteolin glycosides; F5: isoorientin; F6: isoorientin 2''-O-rhamnoside; F7: 3-C-glucoside, 3,4,2',4',6' Pentahydroxychalcone; F8: Isovitexin; F9: Isoscoparin; F10-12: uncharacterised flavonoid peaks; F13: tricin; S L1 = Septoria on flag-leaf; S L2 = Septoria on 2nd leaf; S L3= Septoria on 3rd leaf; M L1 = mildew on flag-leaf; M L2 = mildew on 2nd leaf; M L3 = mildew on 3rd leaf; Lod: stem lodging. See table S31 for Monte Carlo permutation test F and *P* values.

Graphic for the Table of Contents