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Historical changes in the concentrations of selenium in soil and wheat grain from the Broadbalk experiment over the last 160 years

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ABSTRACT

Selenium (Se) intake has decreased substantially in the UK population since 1970s. To investigate whether Se concentration in wheat grain has changed as a result of yield improvement or environmental changes, we analyzed archived wheat grain from the Broadbalk Wheat Experiment at Rothamsted, England, which has been run continuously for over 160 years. Wheat grain and soil samples were selected from plots receiving different fertilizer or manure treatments. Grain Se concentration varied from 11 to 236 ng g^{-1}, with a mean and median of 44 and 32 ng g^{-1}, respectively. Grain samples from the unfertilized control plot had significantly higher concentrations of Se than those from fertilized or manured plots; the latter received various amounts of S and also had higher grain yield. No significant trends in grain Se concentrations were detected in the fertilized or manured plots, in spite of a dramatic increase in grain yield since the introduction of modern short-straw cultivars in the mid 1960s. In the control plot, grain samples had higher Se concentrations in the periods before 1920 or after 1970 than those during 1920–1970. This temporal pattern mirrored that of SO_2 emissions and atmospheric S deposition. Soil Se concentrations showed an increasing trend in all plots over 160 years. The results show that the Se concentration of wheat grain from the Broadbalk experiment was influenced by S inputs from fertilizers and atmospheric deposition, and that improving grain yield through plant breeding has not resulted in a significant decrease in grain Se concentration in the fertilized plots.

1. Introduction

Selenium (Se) is an essential element for human health. Low dietary Se intakes are associated with health disorders including oxidative stress-related conditions, reduced fertility and immune function, and an increased risk of cancers (Rayman, 2000; Rayman, 2002; Whanger, 2004). The recommended intake of Se varies between countries (Thomson, 2004). The US and Canadian recommended dietary allowance (RDA) and the European population reference intake (PRI) are both set at 55 µg day^{-1}, whilst the UK reference nutrient intake (RNI) is 75 and 60 µg day^{-1} for male and female adults, respectively. These recommended levels of intake are set on the basis of the maximization of the activity of the plasma glutathione peroxidase (a Se-containing enzyme). However, there is evidence that Se intakes above these levels may have cancer-preventive benefits (Clark et al., 1996; Clark et al., 1998; Rayman, 2002; Rayman, 2005). Globally, between 0.5 and 1 billion people may have inadequate intakes of Se, and these include populations in the developed countries such as western Europe (Combs, 2001). For instance, Se intake in the UK population has declined from 60 µg day^{-1} in 1974 to less than

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35 µg day^{-1} in 1999, a level that is about half of the UK RNI (Rayman, 2002).

Wheat is the most widely grown food crop with a global production of about 600 million metric tonnes annually (United States Department of Agriculture — National Agricultural Statistics Service, 2004). Wheat and wheat products are an important source of Se in the human diet (Lyons et al., 2003; Hawkesford and Zhao, in press). A dietary survey carried out in the UK in 1995 estimated that cereals and cereal products (mainly wheat) contributed 18–24% to the total Se intake (Ministry of Agriculture Fisheries and Food, 1997). Wheat produced in western and northern Europe generally contains less Se than that produced in North America because of the difference in soil Se status (Hawkesford and Zhao, in press). A recent survey of UK wheat showed a median concentration of 18 ng Se g^{-1} fresh weight (with a 15% moisture content) (Adams et al., 2002), which is approximately one tenth of that reported for US wheat (Wolnik et al., 1983). The recent decline in Se intake in the UK population has been attributed primarily to the replacement of milling wheat imported from North America with that produced in the UK (Broadley et al., 2006; Hawkesford and Zhao, in press). In addition, there is a possibility that Se concentration in wheat may have decreased as a result of the substantial yield improvement over the last few decades. Such a dilution effect has been observed in an experiment comparing 14 US wheat cultivars grown together in a single season (Garvin et al., 2006). However, there has been no report of historical changes in the Se concentration in wheat or other crops grown at the same location. This information is important for assessing if crop Se status has changed as a result of changing cultivars, agronomic inputs or the environment.

The objectives of the present study were to evaluate the long-term trends of Se concentrations in wheat grain under different fertilizer or manure treatments, and to establish whether grain Se concentration has altered as a result of improving yield (e.g. dilution effect) or changes in soil Se concentration. The study was based on the unique archived samples from the Broadbalk Wheat Experiment at Rothamsted, England, which has been continued for over 160 years and is the oldest agricultural experiment in the world.

2. Materials and methods

2.1. *The Broadbalk wheat experiment*

The experiment was set up in 1843 with the original objective of testing the effects of different combinations of inorganic fertilizers and organic manure (farmyard manure, FYM) on yield of winter wheat (*Triticum aestivum*). The experiment has continued up to the present, and has been used in a wide range of agricultural, ecological and environmental studies (Johnston, 1997). The experiment is located in a semi-rural environment at Rothamsted, Hertfordshire, England (longitude 0° 21′ W, latitude 51° 49′ N, elevation 128 m above sea level). The soil is a moderately well drained Aquic Paleudalf with a flinty silty clay loam topsoil overlying chalk parent material. The topsoil contains approximately 26% clay, 53% silt and 21% sand. Soil pH is maintained at 7.0–8.0. Soil organic C varies from 10 g kg^{-1} in the plots receiving no organic manure to 26 g kg^{-1} in the plot receiving annual additions of organic manure. Annual rainfall ranged from 380 to 973 mm (average 686 mm) over the last 150 years, but with no significant long-term trend. The experiment occupies about 5 ha of land, and was originally divided into 17 parallel, main plots (0.24 ha each, later reduced to 0.19 ha with the introduction of 1.5 m paths between plots) for different fertilizer and manure treatments. Winter wheat is usually sown in October–November and harvested in August the following year. Sixteen cultivars have been grown in the experiment (http://www.rothamsted.bbsrc.ac.uk/eRA/), each representing one of the most commonly grown wheat cultivars of its time in England. At harvest every year, samples of grain are oven-dried at 80 °C overnight, and stored in sealed containers in a sample archive. All grain samples selected for this study were visibly intact without any sign of degradation. In this study, we chose grain samples from seven plots representing different fertilizer or manure treatments. Table 1 shows the amounts of fertilizers and manure applied. Some of these treatments have changed little since the earliest years of the experiment, whereas others have had different forms and increasing amounts of N addition in line with increased yield potential. Between 1926 and 1967 the experiment was divided transversely into five sections, and in 1968, further divided into 10 sections, each of which has received different agronomic treatments (continuous wheat, crop rotation, straw incorporation, no herbicide, no fungicide), but the same fertilizer treatment. In this study, soil and wheat grain samples were obtained from whole plots prior to 1926 and from Section I (continuous wheat, no straw incorporation) since 1926. Straw was removed after harvest each year. Forty-one grain samples were taken from each of the plots 3, 7, 9, 10, 14, 15 and 22

Table 1 – Amounts of fertilizers or manure applied to the plots selected for the present study

Plot	Treatment
3	No input (Control) (1844–present)
7	$N_2PKNaMgS$ (1844–1973), N_2PKMgS (1974–2000), N_2KMgS (2001–present)
9	$N^*{}_1PKNaMgS$ (1844–1967), $N_4PKNaMgS$ (1968–1973), N_4PKMgS(1974–2000), N_4KMgS (2001–present)
10	N_2 (1844–2000), N_4 (2000–present)
14	N_2PMgS (1844–1967), N_2PKMgS (1968–2000), N_4PK^* (2001–present)
15	$N_2PKNaMgS$ (1844–1967), $N_3PKNaMgS$ (1968–1984), N_5PKMgS (1985–2000), N_5KMgS (2001–present)
22	FYM (1844–present)

N_1, N_2, N_3, N_4, N_5: 48, 96, 144, 192, 240 kg N ha^{-1}, applied as ammonium sulphate until 1967 (except N^* which was sodium nitrate), as calcium ammonium nitrate from 1968 to 1985, and as ammonium nitrate since 1986.

P: 35 kg P ha^{-1}, applied as superphosphate which also contains 12% S. P fertilization has been stopped since 2001 on Plots 7, 9 and 15 because of the build-up of available P in soil.

K: 90 kg K ha^{-1}, applied as potassium sulfate, or as potassium chloride (K^*).

Mg: 12 kg Mg ha^{-1}, applied as magnesium sulfate until 1973, as Kieserite since 1974.

Na: 16 kg Na ha^{-1}, applied as sodium sulfate.

FYM: 35 t ha^{-1} farmyard manure.

S: varying amounts of S applied, by default, as part of other S-containing fertilizers and FYM.

(Table 1) from the sample archive in 5-yearly intervals for the period of 1845–1965, and at 2- or 3-yearly intervals since 1965. Soils were sampled from the plots periodically during the period 1865 to 2000. The soil samples were air-dried, ground to <2 mm by mortar and pestle before 1965 and thereafter by an iron roller mill, and stored in the archive. Subsamples of soils from 0 to 23 cm depth (the plow layer) were taken for analysis.

2.2. *Analysis of Se in wheat and soil samples*

Approximately 10 g of each grain sample was rinsed briefly with deionized water, dried at 80 °C, and ground to <0.5 mm using a stainless steel centrifugal mill. This milling method was found not to introduce Se contamination in a preliminary study. Soils were ground to <0.15 mm in an agate ball mill. Soil samples (*ca* 0.25 g) were digested with a 5 ml mixture of ultra-pure HNO_3 and $HClO_4$ (85:15 v/v) in a heating block (Zhao et al., 1994). Blanks and a certified reference material (NIST 2711 Montana soil) were included in each batch of digestion. Samples of the ground grain (*ca* 1 g) were digested with ultra-pure HNO_3 (2 ml) and 30% w/v H_2O_2 (2 ml) using a closed-vessel microwave (Mars X, CEM Corp, Matthews, NC), and diluted to 25 ml with ultra-pure (>18 MΩ) deionized water (Adams et al., 2002). The sample vessels were thoroughly acid-washed before use. For quality assurance, a blank and a certified reference material (NIST 1567a wheat flour) were included in each batch of 12 vessels. Concentrations of Se were determined using an Inductively-Coupled Plasma Mass Spectrometer (ICP-MS) equipped with an octopole collision/reaction system (Agilent 7500ce, Agilent Technologies, Palo Alto, CA, USA). Hydrogen gas was used as the reaction gas at 4 ml min^{-1} for eliminating Ar polyatomic interferences at m/z 78 and 80 when measuring Se. Other instrumental conditions were as follows: RF forward power of 1500 W, sample depth of 8 mm from the load coil, carrier gas flow rate of 0.89 L min^{-1} and spray chamber temperature of 2 °C. Calibrations were performed using external standards prepared from 1000 mg L^{-1} single element stock solutions and made up in 5% nitric acid. Two internal standards (In and Y at 100 μg l^{-1}) were used to correct for signal drift. The analytical procedures gave satisfactory results for the two certified reference materials: 1.2±0.2 mg kg^{-1} Se in NIST 1567a wheat flour (certified value 1.1±0.2 mg kg^{-1}) and 1.50±0.11 mg kg^{-1} Se in NIST 2711 Montana soil (certified value 1.54±0.14 mg kg^{-1}).

2.3. *Data analysis*

Biomass yields of grain were retrieved from the electronic Rothamsted archive (http://www.rothamsted.bbsrc.ac.uk/eRA/). The concentrations of Se in grain and soil samples were expressed on a dry weight basis. Analysis of variance (ANOVA) was performed to test the difference among treatments. The data from different years were taken as replicates in ANOVA; this was necessary because the Broadbalk Experiment was started before the advent of modern statistical design and analysis, and the treatments were not replicated. Grain Se data were transformed logarithmically prior to ANOVA to stabilize the variance. Linear regression was used to analyze the temporal trends of Se concentrations in grain and soil. The Genstat software (the 8th Edition, VSN International Ltd, Hemel Hempstead, UK) was used.

3. Results and discussion

3.1. *The range of grain Se concentration in different treatments*

The concentrations of Se in wheat grain from the Broadbalk experiment varied from 11 to 236 ng g^{-1}, with a mean and median of 44 and 32 ng g^{-1}, respectively. Approximately 80% of the samples contained less than 50 ng Se g^{-1}. The nutritional minimum level for animals and humans is about 50–100 ng Se g^{-1} in dry fodder/food, and intake below that may cause Se deficiency (Gissel-Nielsen et al., 1984). Therefore, most of the wheat grown on the Broadbalk experiment over the last 160 years would be considered insufficient to meet human or animal Se requirements. This generally low status of Se is in agreement with results of previous studies of UK crops (Barclay and Macpherson, 1986; Adams et al., 2002).

Fig. 1 shows a boxplot of grain Se concentration in different treatments. It is clear that the control plot (plot 3) had a much larger range of Se concentration than fertilized or manured plots. Analysis of variance based on log-transformed data showed a significant ($P<0.001$) difference among plots. The geometric mean of the Se concentration in grain from the control was about three times larger than that of other treatments. Among the fertilized or manured plots, plots 9, 10 and 22 had significantly ($P<0.05$ based on the least significant difference test) larger geometric means than plots 7, 14 and 15. The results indicate that additions of fertilizer led to a lower concentration of Se in wheat grain. However, long-term additions of organic manure (plot 22) did not necessarily result in a higher concentration of grain Se than inorganic fertilizers.

3.2. *Temporal changes in grain Se concentration*

Fig. 2a and b shows the temporal patterns of grain Se concentrations from the three plots (plot 3 Control, plot 15 NPKMgS and plot 22 FYM). The treatments of plots 3 and 22 have remained unchanged over the entire period of the

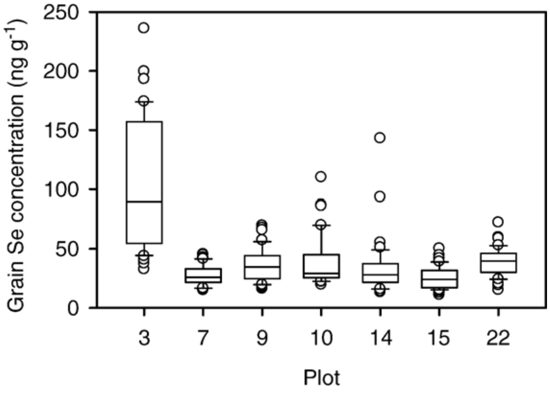

Fig. 1 – Box-plot of Se concentrations in grain from different treatments of the Broadbalk experiment over the last 160 years. The rectangular box represents 25th–75th percentiles, the whiskers represent 10th–90th percentiles, the line inside the box represents the median, and the open circles represent the outliers. See Table 1 for treatments.

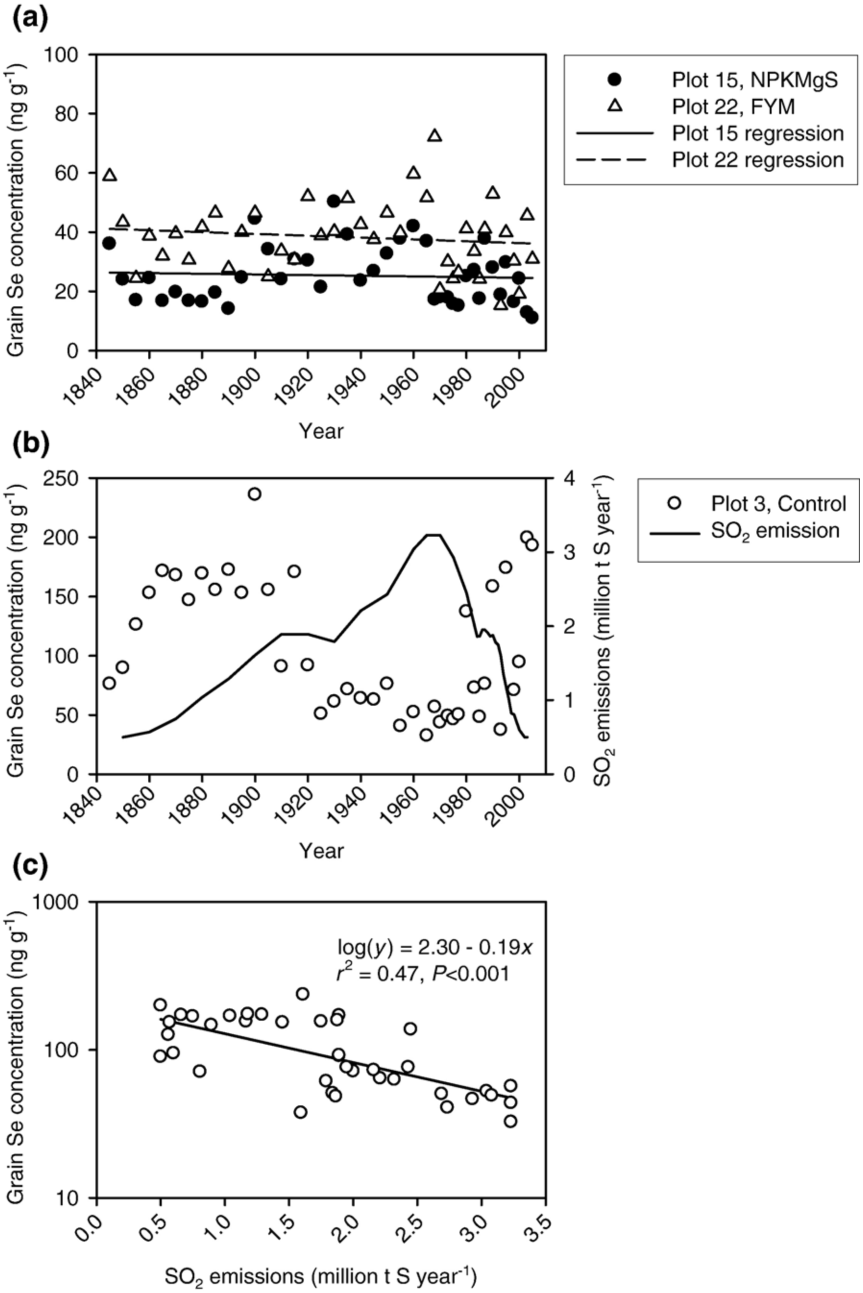

Fig. 2 – The temporal trends in grain Se concentration in plots 15 and 22 (a) and plot 3 (b) of the Broadbalk experiment since 1845, SO_2 emissions in the UK since 1850 (b), and the relationship between log(Grain Se concentration) of the samples from the control plot and UK SO_2 emissions (c). The data for SO_2 are from Zhao et al. (2003).

experiment. The amount of N fertilizer applied to plot 15 has increased from the initial 96 kg ha^{-1} during 1843–1967 to the current level of 240 kg ha^{-1} (Table 1), in line with the increasing yield potential since the introduction of semi-dwarf cultivars in the mid 1960s; yield from this plot broadly reflects the average yield of wheat in England at different times over the last 160 years. There was no significant trend in the concentration of Se in grain from either plot 15 ($P=0.72$) or plot 22 ($P=0.42$); similar patterns were observed in other fertilized plots. In contrast, plot 3 shows an interesting temporal pattern, with higher concentrations in the grain samples before 1920 or after 1970 than those during the period 1920–1970 (Fig. 2b).

Plot 3 is the only plot receiving no S inputs from fertilizers or manures. However, atmospheric deposition of S adds considerable amounts of S to the soil–crop system, with values ranging from below 10 to around 60 kg S ha^{-1} at the experimental site during the last 160 years (Sverdrup et al., 1995; Zhao et al., 2003). The temporal pattern of local S deposition is broadly similar to the SO_2 emissions in the UK over the last 150 years, for which estimates are available (Zhao et al., 2003) and are shown in Fig. 2b. Emissions of SO_2 in the UK increased by more than 6 fold during the period from 1850 to 1965–1970, and since then have decreased rapidly to a present level similar to that in 1850. It appears that the period with low grain Se in plot 3 coincides with that of high S emissions in the UK. Fig. 2c shows that a significant inverse relationship exists between log(Grain Se) in plot 3 and national SO_2 emissions. This relationship is consistent with an antagonistic effect of sulfate on selenate uptake by plants that has been reported in many studies (Terry et al., 2000; Adams et al., 2002). The lower concentrations of Se in grain from fertilized or manured plots than those from the control plot can also be explained, at least partly, by the inputs of S in fertilizers or manures. These fertilizer S inputs could have masked the effect of changing inputs from atmospheric deposition on Se uptake, which is only seen in plot 3.

3.3. *Relationship between grain Se concentration and grain yield*

Another possible reason for the lower concentrations of Se in grain from fertilized or manured treatments compared to that from the control is the effect of dilution resulting from greater yields. Previously, Garvin et al. (2006) showed a negative correlation between grain Se concentrations and grain yield in a set of 14 US wheat cultivars from production eras spanning more than a century, in one of the two experiments in which all of the cultivars were grown side by side in a single season. To examine whether improving grain yield has influenced grain Se concentration in the Broadbalk experiment, log(Grain Se concentrations) were regressed on grain yields (Fig. 3). When data from all seven plots were combined (Fig. 3a), there was a significant ($P<0.001$) negative relationship between grain Se and yield, although r^2 was rather small (0.08). However, when data from the unfertilized control plot (plot 3) were excluded from the regression analysis (Fig. 3b), the relationship between grain Se and grain yield was not significant. Excluding the plot 3 data is appropriate when one wants to examine how improving grain yield through plant breeding over the last century might have influenced grain Se concentration, because grain yield in this unfertilized plot has remained very low over the entire period and has not exhibited a dramatic increase since the mid 1960s, seen in other fertilized plots, as a result of the introduction of modern short-straw cultivars. These analyses suggest that improving grain yield through plant breeding has not resulted in a significant decrease in grain Se concentration.

3.4. *Changes in the concentration of Se in the Broadbalk soils*

Fig. 4 shows the temporal trends in total Se concentrations in the soil from plots 3, 15 and 22; the trends in other plots were similar. In all plots, the soil Se concentration shows an increasing trend

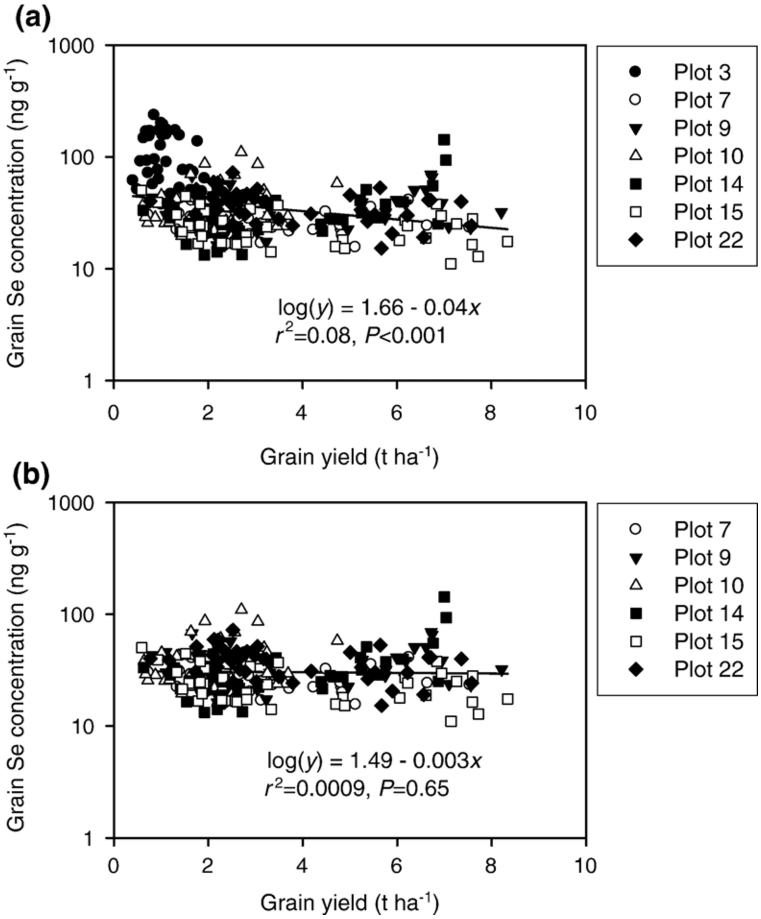

Fig. 3 – The relationship between log(Grain Se concentration) and grain yield in the seven treatments of the Broadbalk experiment selected for this study (a), and in six treatments excluding the data from the unfertilized plot 3 (b). See Table 1 for treatments.

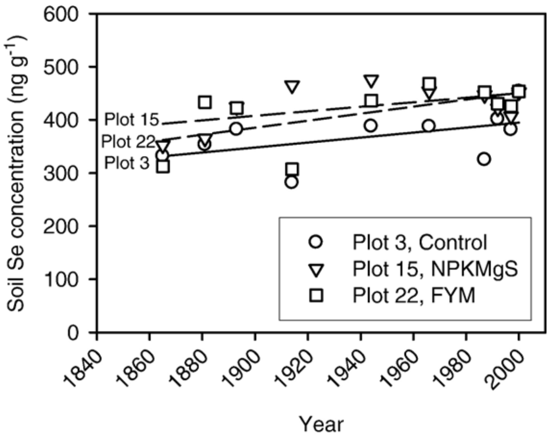

Fig. 4 – The temporal trends in the Se concentration in the topsoils from three treatments of the Broadbalk experiment since 1865.

over the last 160 years. However, these linear trends were not statistically significant ($r^2=0.26–0.37$, $P=0.064–0.13$), partly because of the limited number of soil samples analyzed. The slope of the linear trend varied from 0.43 to 0.85 ng Se g^{-1} per year (equivalent to 1.3–2.5 g Se ha^{-1} per year), with an average of 0.62 ng Se g^{-1} per year. The relative rate of increase is similar to that reported by Haygarth et al. (1993), who studied the long-term effect of atmospheric deposition of Se on soil Se concentrations in several experimental fields at Rothamsted. The increase in soil Se in different plots of the Broadbalk experiment can be attributed to the inputs from atmospheric Se deposition (plot 3) and from the applications of organic manure or inorganic fertilizers. The similar slopes of increase observed in the three plots suggest that either the fertilizers or FYM manure used in the experiment did not add significant amounts of Se to the soil, or that Se from fertilizers and manure was easily lost. Analysis of six FYM samples archived in recent decades (1960–2004) showed total Se in the range of 140–270 ng g^{-1} (mean = 196 ng g^{-1}), which was smaller than the Se concentration in the Broadbalk soil. Atmospheric deposition of Se in the UK is estimated to be approximately 2.2–6.5 g ha^{-1} per year (Fordyce, 2005). The lower net increase of soil Se observed in the Broadbalk soil can be explained by losses of Se by crop uptake, leaching or volatilization. However, the long-term increasing trend in soil Se was not associated with any significant increase in the Se concentration in wheat grain in the present study; possibly because the bioavailable pool of Se in the soil has not increased proportionally, or, any increase in Se uptake by the wheat was negated by the small dilution effect discussed above.

4. Conclusions

This study shows that the Se concentration in the wheat grain produced from Broadbalk has remained very low in the fertilized or manured treatments over the last 160 years, despite an increasing trend of soil Se. The unfertilized plot had a much higher Se status in grain, and its temporal trend appears to be related negatively to the atmospheric S deposition. Sulfur inputs from fertilizers and atmospheric deposition have an important influence on the Se status of wheat grain. Improving grain yield through plant breeding has not resulted in a significant decrease in grain Se concentration in the fertilized plots.

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