Historical trends in iodine and selenium in soil and herbage at the Park Grass experiment, Rothamsted Research, U.K.

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Running Head Title: Historical trends in I and Se. (35 characters max)

Abstract (245 words)

Long term trends in iodine and selenium retention in soil, and uptake by herbage, were investigated in archived samples from the Park Grass Experiment, initiated in 1856 at Rothamsted, UK. Soil (0-23 cm) and herbage samples from plots receiving various mineral fertilisers and organic manures, with and without lime, were analysed for Se and iodine (I) to assess the effect of soil amendment, annual rainfall, crop yield and changes in soil chemistry from 1876 to 2008. Comparing soil from limed and un-limed control (unfertilized) plots, TMAH-extractable Se and I concentrations both diverged, with time, with greater retention in un-limed plots; differences in concentration amounted to 92 and 1660 µg kg^{-1} for Se and I respectively after 105 yr. These differences were broadly consistent with estimated additions from rainfall and dry deposition. Offtake of both elements in herbage was negligible compared to soil concentrations and annual inputs (<0.003% of total soil I and <0.006% of total soil Se). A positive correlation was observed between I and Se concentrations in herbage, suggesting some common factors controlling bioavailability. A growth-dilution effect for I and Se was suggested by the positive correlation between growing season rainfall (GSR) and herbage yield together with soil-to-plant transfer factors decreasing with yield. Phosphate and sulphate fertilizers reduced I and Se herbage concentrations, both through ion competition and increased herbage yield. Results suggest that in intensive agriculture with soil pH control, the I requirement of grazing animals is not likely to be met by herbage alone.

Keywords: Iodine, Selenium, Park Grass, transfer factors, permanent grassland. (6 max)

Introduction

Selenium (Se) and iodine (I) are both essential micronutrients for animal health. Iodine deficiency disorders (IDDs) in cattle and sheep cause impaired fertility and growth problems (Whitehead, 1975; Franke *et al*., 2009; Lidiard, 1995;). Selenium deficiency disorders (SeDDs) often manifest as nutritional muscular dystrophy (white muscle disease), in young animals, which, if untreated (with vitamin E and Se), can be fatal (Fordyce, 2005; Levander, 1986; Oldfield, 1999). Ruminants require Se and I concentrations in feed of approximately 30 - 50 $\mu g\ kg^{-1}$ and 500 $\mu g\ kg^{-1}$ respectively (Levander, 1986; NRC, 2001) but grazed pasture in the UK is often unable to meet these requirements (Lidiard, 1995; Surai, 2006) creating a need for widespread dietary supplementation (Lenz & Lens, 2009; Whitehead, 1979).

Contributions to soil reservoirs of Se and I include inputs from parent material, soil amendments (fertiliser, lime etc.), atmospheric deposition of marine-derived compounds (e.g. methyl iodide; di-methyl selenide) (Haygarth, 1994; Martino et al., 2009) or pollutant aerosols from sources such as coal burning (Fordyce, 2013; Haygarth, 1994; Wu et al., 2014). However, it is widely reported that knowledge of total soil I and Se contents alone does not enable prediction of uptake by vegetation (Hong et al., 2012; Fordyce, 2005; Kashparov et al., 2005;). Soil factors controlling availability to vegetation are broadly similar for both elements. Weak inorganic acid anions (selenite (SeO_3^{2-}), selenate (SeO_4^{2-}), iodide (I^-) and iodate (IO_3^-)) are adsorbed on hydrous oxides of Fe(III), Mn(IV) and Al (Dai et al., 2009; Das et al., 2013). Humus is the main reservoir of Se in many soils, with Se possibly substituted in organic-S compounds (Biederbeck, 1978; Christophersen et al., 2013) and I bound to aromatic carbon moieties (Xu et al., 2011; Yamaguchi et al., 2010); elemental Se in soil has also been reported (Fellowes et al., 2013). The effects of soil pH on adsorption of inorganic I and Se are well understood but the same is not true for the soil characteristics governing transfer between organic and inorganic forms (Dai et al., 2009; Xu et al., 2011). In areas of marginal deficiency, the relative contributions from soil and atmospheric sources to Se and I in pasture vegetation are currently difficult to accurately assess (Haygarth et al., 1995; Watts & Mitchell, 2009; Watts et al., 2010).

The Park Grass experiment at Rothamsted Research, Harpenden, Hertfordshire, U.K. was initiated in 1856. It is the experiment of longest duration on a permanent grassland site in the world (Silvertown et al., 2006). Throughout the history of the trial, samples of herbage and soil of the Batcombe series, a profundic, chromic, endostagnic luvisol (Cranfield University, 2016) have been collected and archived. Soils are currently collected at irregular intervals whereas herbage is harvested twice a year, designated as 'cut 1' and 'cut 2' (Anon, 2006; Silvertown et al., 2006). Selected plots are treated with combinations of N, P, K, Na, Mg and S, farmyard manure (FYM) and pelleted poultry manure according to prescribed regimes (Table 1; Silvertown et al. 2006). Plots established in 1856 were split in 1903 to study the effect of liming: 4.0 t ha^{-1} lime (chalk) was applied every four years. Plots were further split in 1965 into four sub plots (a – d) and lime applied every three years, if necessary, to maintain different soil pH values in each sub-plot: a = pH 7; b = pH 6; c = pH 5 (nominal); d = unlimed (Silvertown et al., 2006). Sub-plot c only achieves the intended pH of 5 when a treatment has an acidifying effect as the natural topsoil pH value at the site is approximately pH 5.5. Two 'control plots' are subjected only to the liming regime described and receive zero fertiliser addition. The Park Grass trial provides a unique situation whereby long-term trends (potentially > 150 yr) in I and Se retention in soil and uptake by herbage under permanent grassland can be examined. Our experimental objectives were therefore to measure soil and herbage I and Se concentrations in archived samples and to use the wider records of the Rothamsted archive to assess the influence of soil amendments, annual rainfall, crop yield and changes in soil chemistry on I and Se dynamics.

Materials and Methods

Sample collection

Samples of soil (0-23 cm) and herbage from harvest dates between 1870 and 2008, and from fertiliser-treatment and control plots (limed and unlimed), were collected from the Rothamsted Archive (Table 2). Temporal changes over the period 1870 – 2008 were investigated on samples from Plot 3 (unfertilised control plot; limed and unlimed) (Table 2). The limed and unlimed treatments are designated 'L' and 'U' (pre-1965) or 'a' and 'd' (post 1965). Four pH regimes were compared using samples collected in 2008. The effect of soil fertiliser amendments were compared using selected samples from 1876 (plots 9, 12, 13 & 14) and 2008 (plots 9/2, 12d, 13/1, 13/2 & 14/2). Where possible, samples of both soil and vegetation (cut 1 only) were collected for each year but not all were available in the archive (Table 2). Despite long periods of sample storage, Haygarth et al. (1993) demonstrated that losses of Se from archived soils by volatilization were undetectable from a comparison of stored subsoil samples (22 – 45 cm) from 1893 and 1987.

Archived air-dry soil samples were milled as required; oven-dried vegetation samples were already milled. Samples of archived liming materials and fertilisers were also analysed to estimate the inputs of I and Se due to soil treatments and liming. These were selected from the earliest and latest years possible. In some cases, only one sample was available, and not all fertilisers used were present in the archive. Samples analysed included: chalk (1972 & 2000), FYM (1981 & 2001), fishmeal (1971 & 1995), K_2SO_4 (1990), poultry manure (2003), $NaNO_3$ (2004), and $Ca(H_2PO_4)_2$ (1938 & 1968). Prior to 1960 slaked lime was also used for liming but no earlier materials were available.

Soil characterisation

Soil pH was measured at Rothamsted Research, initially at a soil:water ratio of 1:5 and, later (from 19590, at a ratio of 1:2.5; the two methods are reported to give similar results (Johnston et al., 1986). Supplementary pH values were obtained from Silvertown et al. (2006). Total soil carbon (SC) and inorganic carbon (SIC) concentrations were measured using an Elemental Analyser (CE Instruments model Flash EA1112), and a Shimadzu TOC-VCPH with a SSM-5000A solids module (Ming, 2004) respectively; soil organic carbon content(SOC) was determined by difference. Olsen-P was measured colorimetrically, following extraction in 0.5 M sodium bicarbonate. Exchangeable cations (Na, Mg, K, Ca) were measured by atomic absorption spectrophotometry after extraction using 1 M ammonium acetate (pH 7).

Determination of iodine and selenium concentrations

All sample dissolution procedures were undertaken in triplicate. Total soil Se content (Se_S) was determined following digestion of 0.2 g (± 0.02 g) soil in 2 mL 70% HNO_3, 1 mL 60% $HClO_4$ and 2.5 mL 70% hydrofluoric acid in perfluoroalkoxy (PFA) digestion vessels (Chilimba et al., 2011). The iodine content of soils ($I_{S\text{-}TMAH}$) was determined following alkaline extraction in tetra methyl ammonium hydroxide (TMAH) (Watts & Mitchell, 2009). Selenium extractable by TMAH ($Se_{S\text{-}TMAH}$) was also determined. Iodine and Se concentrations in organic fertilisers (FYM, poultry manure and fishmeal) and vegetation samples ($I_{V\text{-}TMAH}$ and $Se_{V\text{-}TMAH}$) were measured following TMAH extraction. Total Se content in vegetation (Se_V) was determined following microwave digestion of 0.2 g in 6 mL 70% HNO_3 (Chilimba et al., 2012). Inorganic fertilisers ($NaNO_3$ and K_2SO_4) were dissolved in Milli-Q water (200 mg and 400 mg in 100 ml respectively) prior to analysis. However, superphosphate and chalk present a problem for iodine analysis because they must be dissolved in an acid solution in which iodine is potentially unstable due to formation of volatile I_2. To address this problem we used standard additions of 0, 1, 10, or 15 mg kg^{-1} of Na-iodide or Na-iodate added to samples during acid digestion (Julshamn et al., 2001).

ICP-MS analysis

Analysis of all TMAH extracts was by ICP-MS (Thermo-Fisher Scientific X-series II) in standard mode for iodine and in 'hydrogen cell mode' for Se. Internal standards were Rh, Re and In (10 µg L^{-1}) in a 1 % TMAH matrix with 2% methanol to increase sensitivity for Se analysis (Darrouzès et al., 2007). For materials dissolved in acid (chalk and superphosphate) diluted samples with standard additions were spiked with mix of internal standards (10 µg L^{-1} Rh and Re in Milli-Q water) and analysed for iodine using direct sample aspiration to minimise iodine transfer into the matrix of the sample delivery tubing. The use of external iodine calibration standards gave very similar results to the standard addition approach using iodate- and iodide-spiked samples suggesting losses of iodine from the acid solutions were minimal during sample preparation. Selenium analysis was essentially as described for samples in the TMAH matrix but with external calibration standards and internal standards in 2% nitric acid. Data quality was assessed using certified reference soil and vegetation materials where available, including soils GSS2, GSS5 and GSS6 described in Watts & Mitchell (2009) and tomato leaves (NIST 1573a) for Se. Recoveries of I (soil only) and Se (soil and plant) were > 85% and agreed reasonably with values quoted by Watts & Mitchell (2009).

Iodine and selenium offtake

Yield data from cuts 1 and 2 were obtained from the Rothamsted Electronic Archive (e-RA) to calculate I and Se offtakes (I_{off} or Se_{off}, mg ha^{-1} yr^{-1}) in herbage, shown for I in Eqn. 1:

$$I_{off} = I_{V-TMAH}\,(Y_{cut\,1} + Y_{cut\,2}) \qquad (1)$$

Where $I_{V\text{-}TMAH}$ is the concentration of I in vegetation (µg kg^{-1}) in cut 1 and Y_{cut} represents the yield measured for cut 1 or cut 2 (t ha^{-1} cut^{-1}). Changes in cut 1 harvesting methods meant that post-1960 data for cut 1 had to be corrected to give dry yield values equivalent to pre-1960 values, according to Eqn. 2:

$$Y_{cut\,1} = 0.2743 \text{ x } \left(Y_F^{\ 1.662}\right) \qquad (2)$$

Where Y_F = yield collected by forage harvester, t ha^{-1}. The correction factor was obtained from the relationship between yields for hay and forage harvester cuts (r^2=0.90) for a selection of plots for 1959 and 1992 – 1994 (Electronic Annex 1).

All statistical analyses (linear regression, Pearson correlation analysis and ANOVA) were performed in Minitab (version 16.2.2). Growing season rainfall data was obtained from the electronic Rothamsted Archive (e-RA).

Results and Discussion

Soil iodine and selenium ($I_{S\text{-}TMAH}$, $Se_{S\text{-}TMAH}$ and Se_S)

Soil iodine concentrations ($I_{S\text{-}TMAH}$) were 4.30 – 7.82 mg kg^{-1} (median 5.52 mg kg^{-1}) which is within the range expected for non-coastal UK soils (Johnson, 2003a; Whitehead, 1984) (Electronic Annex 2). Total soil Se concentrations (Se_S) were 430 – 638 µg kg^{-1} (median 485 µg kg^{-1}), typical for the majority of UK soils in that 95% contain <1000 µg kg^{-1} (Broadley et al, 2006) (Electronic Annex 3). Concentrations of TMAH-extractable soil Se ($Se_{S\text{-}TMAH}$) were 352 – 669 µg kg^{-1} (median 412 µg kg^{-1}), which on average accounted for 86% of Se_S (Electronic Annex 3). $Se_{S\text{-}TMAH}$ should include humus-bound Se and reactive inorganic Se associated with Fe, Al and Mn oxides/hydroxides (He et al., 2010). The residual Se (Se_S – $Se_{S\text{-}TMAH}$) may exist bound within mineral structures or as elemental Se (Hurel & Marmier, 2006; Coppin et al., 2009).

There was divergence between limed and unlimed control (unfertilized) plots for both $I_{S\text{-}TMAH}$ and $Se_{S\text{-}TMAH}$ over the course of a century (1903 – 2008) (Figure 1). The limed plots lost soil iodine at a rate equivalent to approximately 1 mg kg^{-1} per century (almost 20% of $I_{S\text{-}TMAH}$) (Figure 1a). Overall, the difference observed between $I_{S\text{-}TMAH}$ inlimed and unlimed plots amounted to 1.66 mg kg^{-1} after 105 years of liming. Volatilization of iodine from stored soil samples cannot be ruled out but this would be greater from acidic soils and so the divergence seen in Fig. 1a is counter to expectation if gaseous losses of I were significant. The concentration of $Se_{S\text{-}TMAH}$ was unchanged in the limed plots over time, but increased in the unlimed plots (Figure 1b, r = 0.914, p = <0.001); the difference in $Se_{S\text{-}TMAH}$ between limed and unlimed control plots was 92 µg kg^{-1} after 100 years. The divergence observed for both Se and I in the limed and unlimed control plots must have arisen from differences between inputs and losses (offtake, volatilization and leaching) over time with the balance favouring greater retention in the soil under acid conditions. These trends are consistent with Se and I accumulation over time due to stronger inorganic adsorption at low pH (Fordyce, 2005; Fleming, 1980; Jacobs, 1989; Neal, 1995) and possibly conversion to humus-bound forms.

Sources of I and Se added to the plots include rainfall, dry deposition and inputs from lime and fertiliser treatments; losses include leaching, offtake in vegetation and volatilization. For 1870 – 2008 inclusive, the estimated average input of I from rainfall was 14.0 g ha^{-1} yr^{-1}, calculated from daily-recorded rainfall and a mean rainfall iodine concentration for the UK of 2 x 10^{-6} g L^{-1} (Hou et al., 2009; Johnson, 2003b; Lidiard, 1995; Neal et al., 2007). Annual Se deposition to the Park Grass site was estimated by Haygarth et al. (1993) to be 0.93 g ha^{-1} yr^{-1} using Se budget models (Haygarth et al., 1991). This value was calculated assuming that dry deposition accounts for 15% of total deposition, based on the average of values from Peirson et al. (1973) and Cawse (1987) who reported that dry deposition accounted for 7% and 24% of total deposition at Wraymires, Cumbria (UK) and Chilton, Oxon, (UK) respectively. A UK average annual Se deposition range of 2.2 – 6.5 g ha^{-1} yr^{-1}, was quoted by Fordyce (2005) and

Broadley et al. (2006), originating from data gathered by Cawse (1980) across five rural UK sites (between 1972 and 1981); however these data do not account for losses from natural systems via leaching, volatilisation etc, unlike the budget models used by Haygarth et al. (1991; 1993). It is also likely that Se deposition was substantially affected by trends in coal burning during the 20th century (Haygarth et al., 1991; Fordyce, 2013). Thus, without reliable measured data it is difficult to estimate the importance of atmospheric Se deposition to the Park Grass site over time except perhaps to within an order of magnitude. Selenium deposition calculated here uses an annual input value of 0.93 g ha^{-1} yr^{-1} as estimated by Haygarth et al (1993) for the Park Grass site

Differences in Se_S between 2008 fertilizer treatment plots were not significant (p = 0.141) but a significant difference was observed in $I_{S\text{-}TMAH}$ (p = <0.001). Iodine and Se concentrations in fertilisers and ground chalk were measured in the samples available from the Rothamsted archive (Electronic Annex 4) and application rates for each sub-plot were used to estimate annual inputs (Table 3). Some fertiliser treatments (Table 1) were unavailable, including sodium and magnesium sulphates (plots 9/2 and 14/2) and ammonium sulphate (plot 9/2), and therefore their contributions could not be assessed. Thus, annual inputs of I from chalk (pH 7 sub-plots) and fertilizer were calculated to be a *minimum* of ~4.5 – 8.8 g ha^{-1} yr^{-1} and ~2.2 – 5.1 g ha^{-1} yr^{-1} respectively, similar in magnitude, when combined, to inputs from aerial deposition (14 g ha^{-1} yr^{-1}). There was no clear evidence of an historic imprint from fertilizer treatments (Table 3) with the possible exception of Plot 14/2 which had elevated I concentrations. It is suspected that this may be due to past applications of Chilean nitrate (now sodium nitrate) which is known to have large concentrations of I (Eriksen, 1981). Selenium was similar in that fertilizer inputs (0.546 – 1.26 g ha^{-1} yr^{-1}) were comparable to those expected from aerial deposition; inputs of Se from chalk (0.027 – 0.053 g ha^{-1} yr^{-1} for pH 7 sub-plots) were much lower (Table 3).

Iodine and selenium in herbage (I_V, Se_V, I_{TF}, Se_{TF})

Differences in $I_{V\text{-}TMAH}$ ($p = <0.001$) and iodine transfer factor (I_{TF}) ($p = <0.005$) between treatment plots were highly significant. Values of $I_{V\text{-}TMAH}$ were 112 - 285 μg kg^{-1}, with median values of 172 μg kg^{-1} (control plot 3, pre-2008, U), 198 μg kg^{-1} (control plot 3, pre-2008, L) and 146 μg kg^{-1} (2008 samples); a significant difference was observed in $I_{V\text{-}TMAH}$ between these three groups of samples (ANOVA, $p = 0.002$). Thus herbage iodine concentrations fell well below optimum dry matter concentrations required for adequate nutrition of beef cattle (400 μg kg^{-1}) or dairy cattle (500 μg kg^{-1}) (NRC, 2001).

Differences in Se_V and Se transfer factor (Se_{TF}) were also highly significant ($p = <0.005$ and $p = <0.001$, respectively). Values of Se_V were 12.0 – 56.5 μg kg^{-1}, with median values of 43.4 μg kg^{-1} (control plot 3, pre-2008, U), 38.1 μg kg^{-1} (control plot 3, pre-2008, L) and 22.2 μg kg^{-1} (2008 samples). These values are similar to the range for Se_V (32 - 78 μg kg^{-1}) reported by Haygarth et al. (1993) for Park Grass herbage between 1861 and 1990. The Se concentrations in all but one of the control plot herbage samples met the required levels for adequate ruminant nutrition (30-50 μg kg^{-1}; Levander, 1986) but herbage in the fertilizer (2008) plots fell below this limit. For TMAH extraction of herbage, values of $Se_{V\text{-}TMAH}$ were 12.1 – 47.5 μg kg^{-1}, with median values of 37.8 μg kg^{-1} (control plot 3, pre-2008, U), 38.2 μg kg^{-1} (control plot 3, pre-2008, L) and 21.7 μg kg^{-1} (2008 samples). Correlation of Se_V and $Se_{V\text{-}TMAH}$ ($r = 0.935$) showed that $Se_{V\text{-}TMAH}$ was 90.1% of Se_V on average, suggesting that TMAH extraction provides a reasonable estimate of the Se content of plant material. When all samples were considered there were significant positive correlations between $I_{V\text{-}TMAH}$ and Se_V (Figure 2; $r = 0.732$; $p = <0.001$), and for comparisons of control and treatment (2008) plots ($r = 0.476$, and $r = 0.826$, respectively (Table 4)). This suggests that the bioavailability of both elements to grass may be controlled by similar factors.

Soil-to-plant transfer of I and Se

When all samples were considered together there was a significant positive correlation between $I_{S\text{-}TMAH}$ and $I_{V\text{-}TMAH}$ ($r = 0.464$), also for treatment plots (2008) considered separately ($r = 0.474$) but not for control plots. Previous studies have observed a relationship between soil and vegetation iodine (Dai et al., 2006; Hong et al., 2012; Weng et al., 2008) but the poor correlation seen here suggests that other factors also influenced $I_{V\text{-}TMAH}$. Plots 14/2 and 9/2, both with large inorganic fertiliser inputs (Table 1) and higher yields than control plots, produced lower values of $I_{V\text{-}TMAH}$ compared to the general trend with $I_{S\text{-}TMAH}$ (Electronic Annex 1). By contrast, for Se, there was a negative correlation ($p < 0.05$) between $Se_{S\text{-}TMAH}$ and $Se_{V\text{-}TMAH}$ ($r = -0.457$) for plot 3 (control) data. The lower TMAH-extractability of soil Se at high pH coupled with greater Se availability to plants under alkaline conditions (Chilimba et al., 2012) may have contributed to this. However, a similar negative correlation exists between Se_S and Se_V ($r = -0.555$), which cannot be explained in those terms as soil pH does not have a significant effect on total Se accumulation (Se_S). Total concentration of Se in soil is generally considered to be a poor predictor of associated vegetation Se concentrations (Chilimba et al., 2012; Fordyce, 2005).

There were no significant correlations between $I_{V\text{-}TMAH}$ or Se_V with SOC, Olsen P or exchangeable cations. A significant negative correlation was observed in the control plots between soil pH and $I_{V\text{-}TMAH}$ ($r = -0.47$). A significant difference in $I_{V\text{-}TMAH}$ between limed and unlimed plots was also observed (ANOVA, $p = 0.038$). No correlation was observedin control plots between soil pH and Se_V. No correlation was observed between soil pH and $I_{V\text{-}TMAH}$ or Se_V in the treatment plots.

Effect of yield on I and Se uptake

Highly significant negative correlations were observed between annual yield (Y) and transfer factors (mg kg^{-1} herbage /mg kg^{-1} soil) for both I ($r = -0.598$) and Se ($r = -0.621$) when treatment and control plots were considered together (Figures 3a & b), suggesting a 'yield dilution' effect. Bioavailable I and Se are present as inorganic species in soil solution and are replenished by desorption of inorganic forms from the solid phase, organic matter decomposition and aerial inputs (Dai et al., 2009; Landini et al., 2011; Shetaya et al., 2012). Thus, the trends in Figure 3 suggest that the growth of herbage can be sufficiently rapid to outstrip the replenishment rate. Smith et al. (1999) observed this relationship in pasture growth, where slower winter growth was associated with greater iodine concentrations. However, when control plots alone were considered (Plot 3), the correlations between yield and transfer factor were not significant for either element. Thus, the apparent yield dilution effect observed when fertilized plots were included may actually result from specific effects of the phosphate and sulphate fertilisers applied to treatment plots. Highly significant negative correlations were observed between both $I_{V\text{-}TMAH}$ and Se_V with sulphate fertiliser (I, $r = -0.831$, $P < 0.001$; Se, $r = -0.893$, $P = <0.001$) and phosphate fertiliser (I, $r = -0.615$, $P = <0.005$; Se, $r = -0.756$, $P = <0.005$) inputs, when control plots (Plot 3, 2008) and treatment plots which did not receive organic fertiliser inputs (Plots 9/2 and 14/2) were considered together (Figure 4a & 4b). It is recognised that sulphate ions suppress uptake of Se (Fordyce, 2005). Similarly, Fan et al. (2008) investigated changes in Se concentrations in archived soil and wheat grain samples from the Broadbalk Wheat Experiment at Rothamsted and observed that inputs of S from fertilizers and atmospheric deposition was the main influence on wheat grain concentration. Phosphate may act as a competitor ion for soil adsorption sites (He et al., 1994, 2010), effectively increasing iodide and selenite solubility but may also increase plant growth causing a dilution effect for I and Se (Fordyce, 2005; Jacobs, 1989; Mayland, 1994; Neal, 1995). Figures 4a and 4b also show that $I_{V\text{-}TMAH}$ and Se_V in control samples from 2008 are comparable to the long term average in limed (sub-plot a) and unlimed (sub-plot d) control samples, particularly in the case of Se.

In agreement with published literature (e.g. Silvertown et al., 2006; Tilman et al., 1994) there was a significant positive correlation between herbage yield (Y) and 'growing season rainfall' (GSR, mm) (1870 – 2008) for the control plots ($r = 0.524$, $p < 0.001$). GSR was nominally defined as precipitation between February 1st and the date of the first cut each year, calculated from the sum of daily rainfall recorded. The effect of GSR can only be considered for control plots, as the treatment plot samples were all from 2008. A significant positive correlation between GSR and $I_{V\text{-}TMAH}$ was observed ($r = 0.349$, $p = 0.003$) but no correlation was seen for Se_V.

Offtake of Se and I

Estimated iodine offtake was extremely small compared to both $I_{S\text{-}TMAH}$ (< 0.003% of $I_{S\text{-}TMAH}$) and the annual inputs (Table 3). For the treatment plots, I_{off} was heavily influenced by the increased yield resulting from fertiliser addition. The combined effect of the positive correlation of GSR on yield, yield on $I_{V\text{-}TMAH}$ (possible negative correlation due to dilution), and GSR on $I_{V\text{-}TMAH}$ (positive correlation assuming inputs of I from rainfall and *amount* of rainfall are positively correlated) resulted in an overall significant positive correlation between GSR and iodine offtake, I_{off}, ($r = 0.641$, $p < 0.001$). The estimated amount of iodine provided by rainfall greatly exceeded the amount removed in vegetation: rainfall I input was estimated to be between 11.4 and 79.5 (median 27.6) times greater than I_{off} for control plot 3 vegetation samples over the period 1870 – 2008 inclusive. Selenium offtake (Table 3) was also very small compared to annual inputs and was negligible compared to soil reserves.

Conclusions

In *unlimed* control plots both $I_{S\text{-}TMAH}$ and $Se_{S\text{-}TMAH}$ increased over time at a rate consistent with calculated inputs from aerial deposition and there was divergence with *limed* plots, accentuated for iodine (but not Se) by a steady long term loss from the *limed* control soils. Thus soil pH was the dominant factor controlling accumulation of both $I_{S\text{-}TMAH}$ and $Se_{S\text{-}TMAH}$ ($p = <0.001$ in both instances). The trend for both I and Se in soil over a century of consistent management underlines the length of time required to reach steady state conditions under constant agronomic practice.

Iodine concentration in herbage ($I_{V\text{-}TMAH}$) was positively correlated with $I_{S\text{-}TMAH}$, suggesting that soil iodine is an important source of I uptake. However, rainfall may be a more important source as estimated inputs greatly exceeded offtake and a significant correlation between GSR and $I_{V\text{-}TMAH}$ was observed. This may suggest that biofortification of crops with I would be more effective if delivered as frequent applications in liquid form (eg 'fertigation' in irrigation water, Cao et al., (1994)) rather than a single basal fertiliser dressing. An even stronger correlation between GSR and I_{off} reflects the yield enhancement of greater GSR. Off-take by the herbage had a negligible effect on $I_{S\text{-}TMAH}$. Uptake of Se by plants was negatively correlated with addition of sulphate and phosphate fertilisers (as was I), and below requirements for ruminant nutrition, due to suppression of uptake and enhanced vegetation yields leading to dilution of Se concentration in plant material. This also occurred in the FYM treated plots.

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Reference List

Anon (2006) Guide to the Classical and other Long-term Experiments, Datasets and Sample Archive. Lawes Agricultural Trust Co Ltd, ISBN 0951445693, 20-31.

Biederbeck, B.P. 1978. Soil organic sulphur and fertility. In: *Soil Organic Matter* (eds. M.Schnitzer and S.Kahn) Elsevier, New York, USA, pp. 273-310.

Broadley, M.R., White, P.J., Bryson, R.J., Meacham, M.C., Bowen, H.C., Johnson, S.E., Hawkesford, M.J., McGrath, S.P., Zhao, F.J., Breward, N., Harriman, M. & Tucker, M. 2006. Biofortification of UK food crops with selenium. *Proceedings of the Nutrition Society*, **65**, 169-181.

Cao, X.Y., Jiang, X.M., Kareem, A. et al. (1994). Iodination of irrigation water as a method of supplying iodine to a severely iodine deficient population in Xinjiang, China. Lancet 344, 107 – 110.

Cawse, P.A. 1980. Deposition of trace elements from the atmosphere in the UK. In: MAFF Reference Book 326, Inorganic Pollution and Agriculture. HMSO, London.

Cawse, P.A. 1987. Trace and major elements in the atmosphere at rural locations in Great Britain, 1972-1981. In: *Pollutant Transport and Fate in Ecosystems* (eds. P.J.Coughtrey, M.H.Martin & M.H.Unsworth) Blackwell, Oxford, England, pp. 89-112.

Chilimba, A.D.C., Young, S.D., Black, C.R., Rogerson, K.B., Ander, E.L., Watts, M.J., Lammel, J. and Broadley, M.R. 2011. Maize grain and soil surveys reveal suboptimal dietary selenium intake is widespread in Malawi. Scientific Reports 1, 72. *Published online* DOI: 10.1038/srep00072

Chilimba, A.D.C., Young, S.D., Black, C.R., Meacham, M.C., Lammel, J., Broadley, M.R. 2012. Agronomic biofortification of maize with selenium (Se) in Malawi. *Field Crops Research*, **125**, 118-128.

Christophersen, O.A., Lyons, L.G., Haug, A. and Steinnes, E. 2013. Selenium pp 429 - 463 in Alloway, B.J. (Ed) Heavy Metals in Soil. Springer, Dordrecht.

Coppin, F., Chabroullet, C., Martin-Garin, A. 2009. Selenite interactions with some particular organic and mineral fractions isolated from a natural grassland soil. *European Journal of Soil Science*, **60**, 369-376.

Cranfield University. 2016. *The Soils Guide.* Available: www.landis.org.uk. Cranfield University, UK. Last accessed 25/07/2016

Dai, J.L., Zhu, Y.G., Huang, Y.Z., Zhang, M. & Song, J.L. 2006. Availability of iodide and iodate to spinach (Spinacia oleracea L.) in relation to total iodine in soil solution. *Plant and Soil,* **289,** 301-308.

Dai, J.L., Zhang, M., Hu, Q.H., Huang, Y.Z., Wang, R.Q. & Zhu, Y.G. 2009. Adsorption and desorption of iodine by various Chinese soils: II. Iodide and iodate. *Geoderma,* **153,** 130-135.

Darrouzès J. Bueno M., Lespès G., Holeman M.,& Potin-Gautier M. 2007. Optimisation of ICPMS collision/reaction cell conditions for the simultaneous removal of argon based interferences of arsenic and selenium in water samples. *Talanta,* **71**, 2080-2084.

Das, S., Hendry, J.M., Essilfie-Dughan, J. 2013. Adsorption of selenate onto ferrihydrite, goethite, and lepidocrocite under neutral pH conditions. *Applied Geochemistry*, **28**, 185-193.

Ericksen G.E. 1981, Geology and Origin of the Chilean Nitrate Deposits. *United States Geological Survey Professional Paper* 1188.

Fan M-S., Zhao F-J., Poulton P.R. & McGrath S.P. (2008). Historical changes in the concentrations of selenium in soil and wheat grain from the Broadbalk experiment over the last 160 years. *Science of the Total Environment*. **389**, 532-538.

Fellowes J.W., Pattrick R.A.D., Boothmana C., Al Lawatia W.M.M., van Dongen B.E., Charnock J.M., Lloyd J.R. & Pearce C.I. 2013. Microbial selenium transformations in seleniferous soils. *European Journal of Soil Science*, **64**, 629-638.

Fleming, G.A. 1980. Essential Micronutrients 11: Iodine and Selenium. In: *Applied Soil Trace Elements* (ed B.E.Davis). New York, Wiley, pp. 199-234.

Fordyce, F. 2005. Selenium deficiency and toxicity in the environment. In: *Essentials of Medical Geology: Impacts of the Natural Environment on Public Health* (eds. O.Selinus & B.J.Alloway). Academic Press, pp. 373-415.

Fordyce F. M. (2013). "Selenium deficiency and toxicity in the environment," in *Essentials of Medical Geology* ed. Selinus O., editor. (Berlin: Springer) 375–416.

Franke, K., Meyer, U.,Wagner, H., Hoppen, H.O. and Flachowsky, G. 2009. Effect of various iodine supplementations, rapeseed meal application and two different iodine species on the iodine status and iodine excretion of dairy cows. Livestock Science 125, 223 – 231.

Haygarth, P.M., Jones, K.C. & Harrison, A.F. 1991. Selenium cycling through agricultural grasslands in the UK: budgeting the role of the atmosphere. *The Science of the Total Environment*, **103,** 89-111.

Haygarth, P.M., Cooke, A.I. & Jones, K.C. 1993. Long-term change in the biogeochemical cycling of atmospheric selenium: Deposition to plants and soil. *Geophysical Research*, **98**, 16,769-16,776.

Haygarth, P.M. 1994. Global importance and cycling of selenium. In: *Selenium in the Environment* (eds. W.T.Frankenburger & S.Benson). Marcel-Dekker, New York, pp. 1-28.

Haygarth P.M., Harrison A.F. & Jones K.C. 1995. Plant selenium from soil and the atmosphere. *Journal of Environmental Quality*, **24(4)**, 768-771.

He, Z.L., Shentu, J. & Yang, X.E. 2010. Chemical Behaviour of Manganese and Selenium in Soils. In: Trace Elements in Soils (ed. P.S.Hooda). Wiley, Chichester, United Kingdom, pp. 481-495.

He, Z.L., Yang, X.E., Zhu, Z.X., Zhang, Q., Xia, W. & Tan, J. 1994. Effect of phosphate on the sorption, desorption and plant-availability of selenium in soil. *Fertiliser research*, **39**, 189-197.

Hong, C., Weng, H., Jilani, G., Yan, A., Liu, H. & Xue, Z. 2012. Evaluation of iodide and iodate for adsorption-desorption characteristics and bioavailability in three types of soil. *Biological Trace Element Research,* **146,** 262-271.

Hou, X.L., Hansen, V., Aldahan, A., Possnert, G., Lind, O.C. & Lujaniene, G. 2009. A review on speciation of iodine-129 in environmental and biological samples. *Analytica Chimica Acta,* **632,** 181-196.

Hurel, C. & Marmier, N. 2006. Sorption of Selenium (IV) on Mineral Mixtures: Role of Minor Phases in the Modelling Part. MRS Proceedings, **932**, 13.1. Published online DOI: 10.1557/PROC-932-13.1.

Jacobs, L.W. 1989. Selenium in agriculture and the environment. *Soil Science Society of America Special Publication no. 23*. Madison, W I : American Society of Agronomy and Soil Science Society of America.

Johnson, C.C. 2003a. Database of the iodine content of soils populated with data from published literature. British Geological Survey Commissioned Report.

Johnson, C.C. 2003b. The geochemistry of iodine and its application to environmental strategies for reducing the risks from iodine deficiency disorders (IDD). British Geological Survey Commissioned Report.

Johnston, A.E., Goulding, K.W.T. & Poulton, P.R. 1986. Soil acidification during more than 100 years under permanent grassland and woodland at Rothamsted. *Soil use and management*, **2**, 3-10.

Julshamn, K., Dahl, L. & Eckhoff, K., 2001. Determination of iodine in seafood by inductively coupled plasma/mass spectrometry. *Journal of AOAC International*, **84**, 1976-1983.

Kashparov, V., Colle, C., Zvarich, S., Yoschenko, V., Levchuk, S. & Lundin, S. 2005. Soil-to-plant halogens transfer studies 1. Root uptake of radioiodine by plants. *Journal of Environmental Radioactivity,* **79,** 187-204.

Landini, M., Gonzali, S. & Perata, P. 2011. Iodine biofortification in tomato. *Journal of Plant Nutrition and Soil Science,* **174,** 480-486.

Lenz M. & Lens P.N.L. 2009. The essential toxin: The changing perception of selenium in environmental sciences. *Science of the Total Environment*. **407**, 3620-3633.

Levander, O.A. 1986. Selenium. In: *Trace elements in human and animal nutrition(Fifth Edition)* (ed. W.Mertz). Academic Press, New York, pp. 209-279.

Lidiard, H.M. 1995. Iodine in the reclaimed upland soils of a farm in the Exmoor national-park, Devon, UK and its impact on livestock health. *Applied Geochemistry*, **10(1)**, 85-95.

Martino, M., Mills, G.P., Woeltjen, J. & Liss, P.S. 2009. A new source of volatile organoiodine compounds in surface seawater. *Geophysical Research Letters,* **36**, 1-5.

Mayland, H.F. 1994. Selenium in plant and animal nutrition. In: Selenium in the Environment (eds. W.T.Frankenberger Jr & S.Benson). Marcel Dekker, New York, pp. 29-46.

Ming, C.A. 2004. Total organic carbon in soil - SSM5000A application note, Shimadzu.

Neal, R.H. 1995. Selenium. In: *Heavy Metals in Soils* (ed. B.J.Alloway). London, Blackie Academic & Professional, pp. 260-283.

Neal, C., Neal, M., Wickham, H., Hill, L. & Harman, S. 2007. Dissolved iodine in rainfall, cloud, stream and groundwater in the Plynlimon area of mid-Wales. *Hydrology and Earth System Sciences,* **11,** 283-293.

NRC (2001): Nutrient Requirement in Dairy Cattle. 7th Ed., Nat. Acad. Press, Washington, D.C.

Oldfield, J.E. 1999. Selenium World Atlas. Selenium-Tellurium Development Association, Belgium, pp. 38.

Peirson, D.H., Cawse, P.A., Salmon, L. & Cambray, R.S. 1973. Trace Elements in the Atmospheric Environment. *Nature*, **241**, 252-256.

Shetaya, W.H., Young, S.D., Watts, M.J., Ander, E.L. & Bailey, E.H. 2012. Iodine dynamics in soils. *Geochimica et Cosmochimica Acta,* **77,** 457-473.

Silvertown, J., Poulton, P., Johnston, E., Edwards, G., Heard, M. & Biss, P.M. 2006. The Park Grass Experiment 1856-2006: Its contribution to ecology. *Journal of Ecology,* **94,** 801-814.

Smith, L.C., Morton, J.D. & Catto, W.D. 1999. The effects of fertiliser iodine application on herbage iodine concentration and animal blood levels. *New Zealand Journal of Agricultural Research,* **42,** 433-440.

Surai, P.F. 2006. In: *Selenium in Nutrition and Health*. Nottingham University Press, United Kingdom, pp. 589-628.

Tilman, D., Dodd, M.E., Silvertown, J., Poulton, P.R., Johnston, A.E. & Crawley, M.J. 1994. The Park Grass Experiment: Insights from the most long-term ecological study. In: *Long-term experiments in agricultural and ecological sciences* (eds. R.A.Leigh & A.E.Johnston). Wallingford, CAB International, pp 287-303.

Warren, R.G. & Johnston, A.E. 1964. The Park Grass Experiment. Rothamsted Experimental Station Report for 1963, 240–262.

Watts, M.J. & Mitchell, C.J. 2009. A pilot study on iodine in soils of Greater Kabul and Nangarhar provinces of Afghanistan. *Environmental Geochemistry and Health,* **31,** 503-509.

Watts, M.J., O'Reilly, J., Maricelli, A., Coleman, A., Ander, E.L., Ward, N.I. 2010. A snapshot of environmental iodine and selenium in La Pampa and San Juan provinces of Argentina. *Journal of Geochemical Exploration*, **107(2)**, 87-93.

Weng, H.X., Weng, J.K., Yan, A.L., Hong, C.L., Yong, W.B. & Qin, Y.C. 2008. Increment of iodine content in vegetable plants by applying iodized fertilizer and the residual characteristics of iodine in soil. *Biological Trace Element Research,* **123,** 218-228.

Whitehead, D.C. 1975. Uptake of perennial pyegrass of iodide, elemental iodine and iodate added to soil as influenced by various amendments. *Journal of the Science of Food and Agriculture,* **26,** 361-367.

Whitehead, D.C. 1979. Iodine in the environment with particular reference to agriculture. *Journal of Applied Ecology,* **16,** 269-279.

Whitehead, D.C. 1984. The distribution and transformations of iodine in the environment. *Environment International,* **10,** 321-339.

Wu, D., Du, J., Deng, H., Wang, W., Xiao, H. & Li, P. 2014. Estimation of atmospheric iodine emission from coal combustion. *International Journal of Environmental Science and Technology*, **11**, 357-366.

Xu, C., Miller, E.J., Zhang, S., Li, H.-P., Ho, Y.-F., Schwehr, K.A., Kaplan, D.I., Otosaka, S., Roberts, K.A., Brinkmeyer, R., Yeager, C.M. & Santschi, P.H. 2011. Sequestration and remobilization of radioiodine (^{129}I) by soil organic matter and possible consequences of the remedial action at Savannah River Site. *Environmental Science & Technology,* **45,** 9975-9983.

Yamaguchi, N., Nakano, M., Takamatsu, R. & Tanida, H. 2010. Inorganic iodine incorporation into soil organic matter: evidence from iodine K-edge X-ray absorption near-edge structure. *Journal of Environmental Radioactivity,* **101,** 451-457.

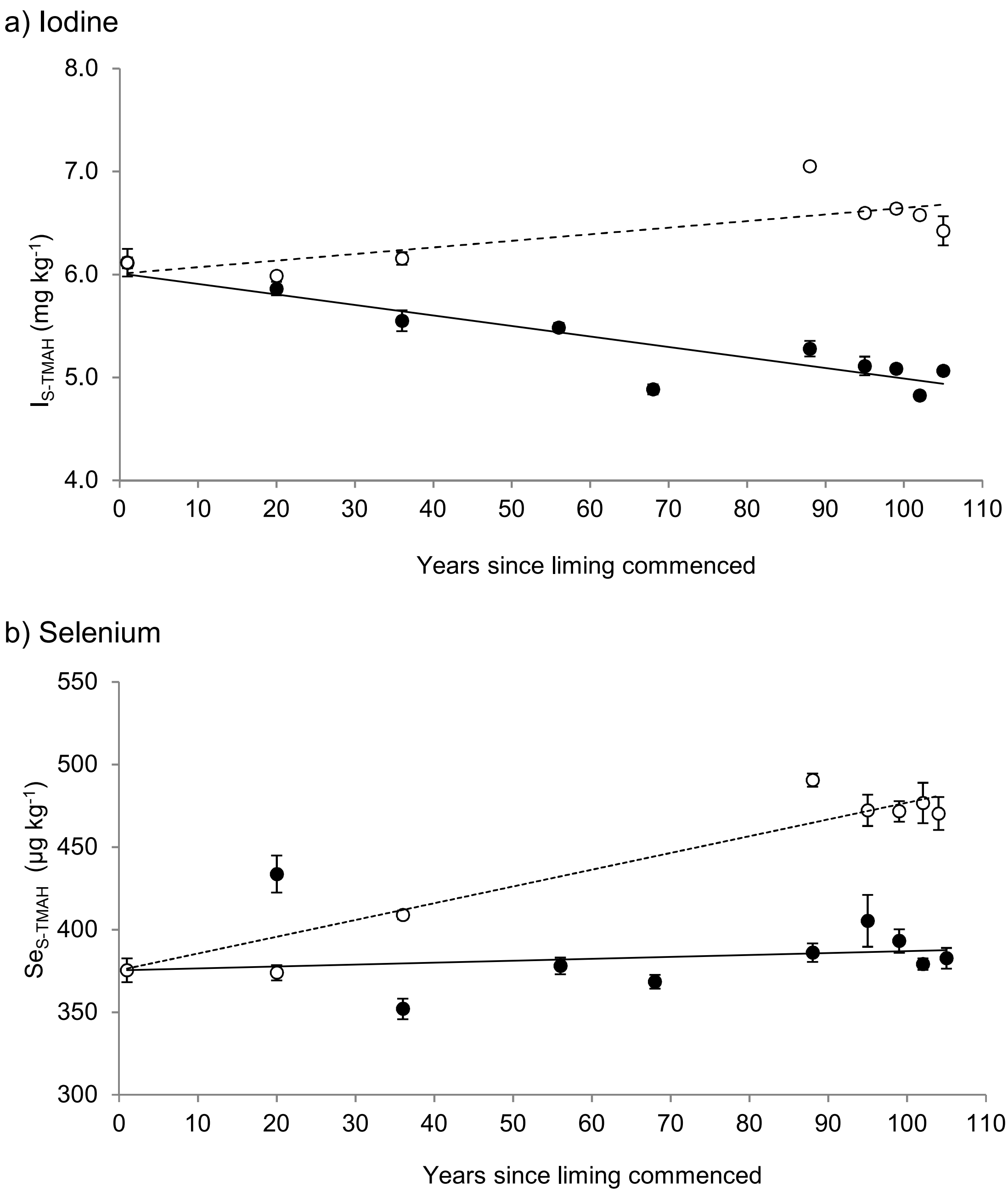


Figure 1: Concentrations of TMAH-extractable iodine ($I_{S\text{-}TMAH}$) (a) and selenium ($Se_{S\text{-}TMAH}$) (b) in Plot 3 (control) soils after liming commenced in 1903. Data are shown for unlimed (○; dashed regression line) and limed treatments (●; solid regression line). Error bars show the standard error of 3 replicate analyses.

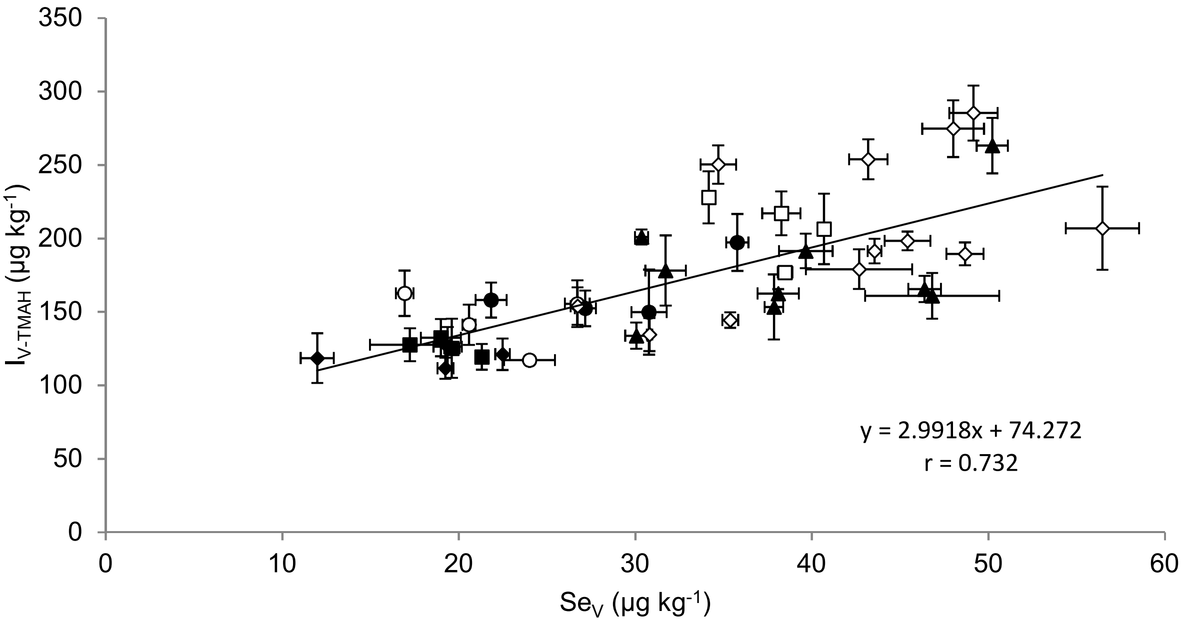
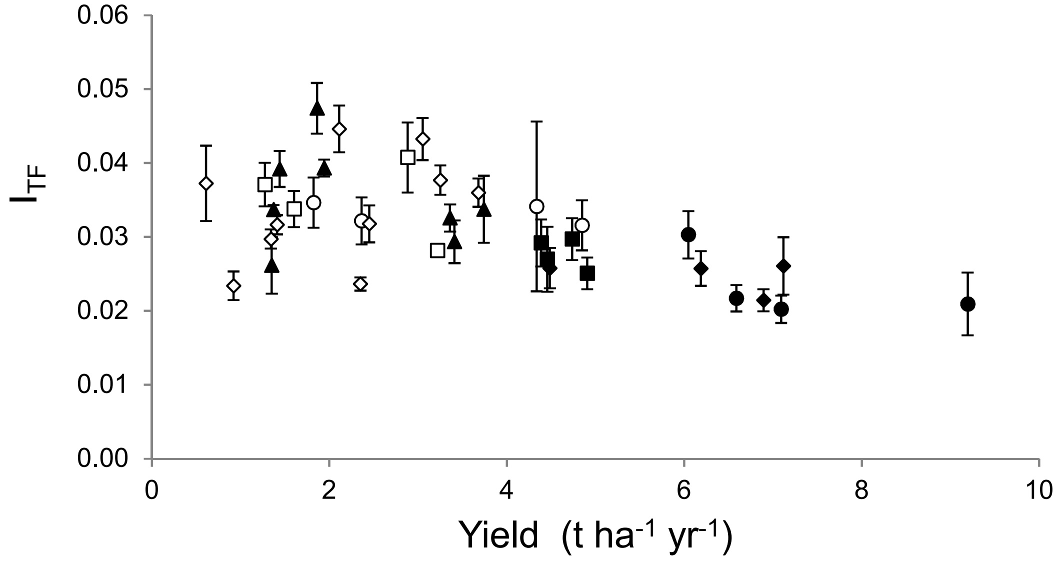


Figure 2: Relationship between iodine and selenium concentrations in herbage samples from Plot 3 (control), 2008 (□); Plot 9/2, 2008 (◆); Plot 13/1, 2008 (○); Plot 13/2, 2008 (■); Plot 14/2, 2008 (●);Plot 3 (control), pre-2008 unlimed (◇) and Plot 3 (control), pre-2008 limed (▲) samples. Error bars show the standard error of 3 replicate analyses.

a) Iodine



b) Selenium

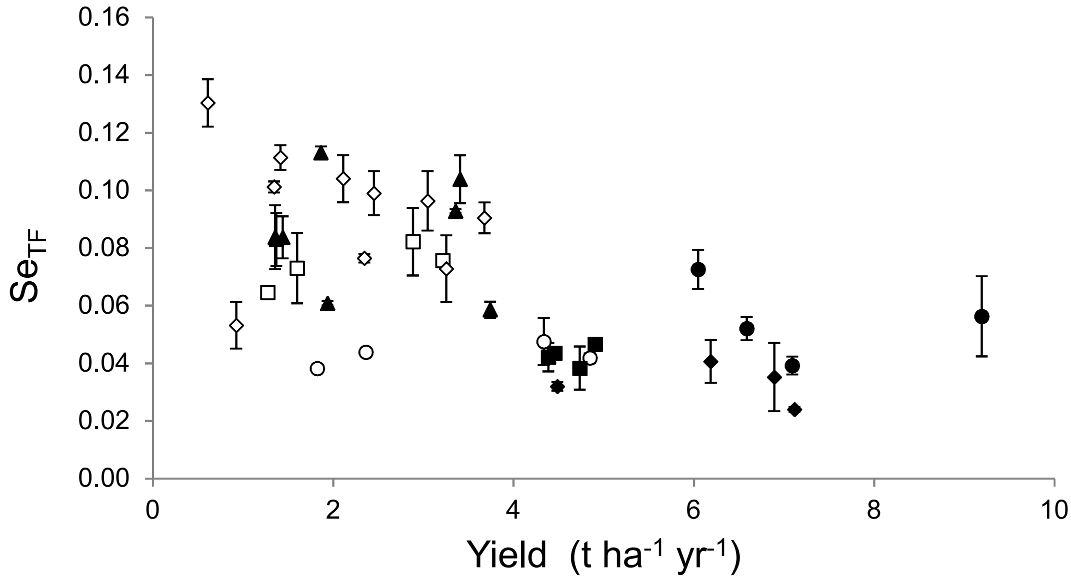
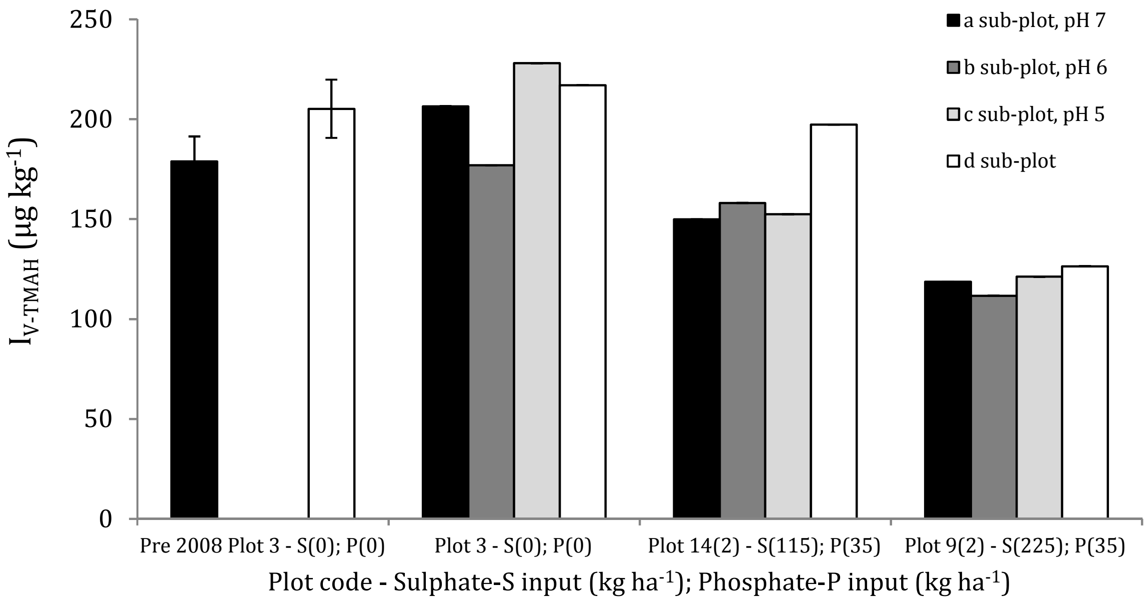


Figure 3: Relationship between vegetation yield and soil-to-vegetation transfer factor for iodine (a, I_{TF}) and selenium (b, Se_{TF}) in samples from Plot 3 (control), 2008 (□); Plot 9/2, 2008 (◆); Plot 13/1, 2008 (○); Plot 13/2, 2008 (■); Plot 14/2, 2008 (●);Plot 3 (control), pre-2008 unlimed (◇) and Plot 3 (control), pre-2008 limed (▲) samples. Error bars show the standard error of 3 replicate analyses.

a) Iodine ($I_{V\text{-}TMAH}$)



b) Selenium (Se_V)

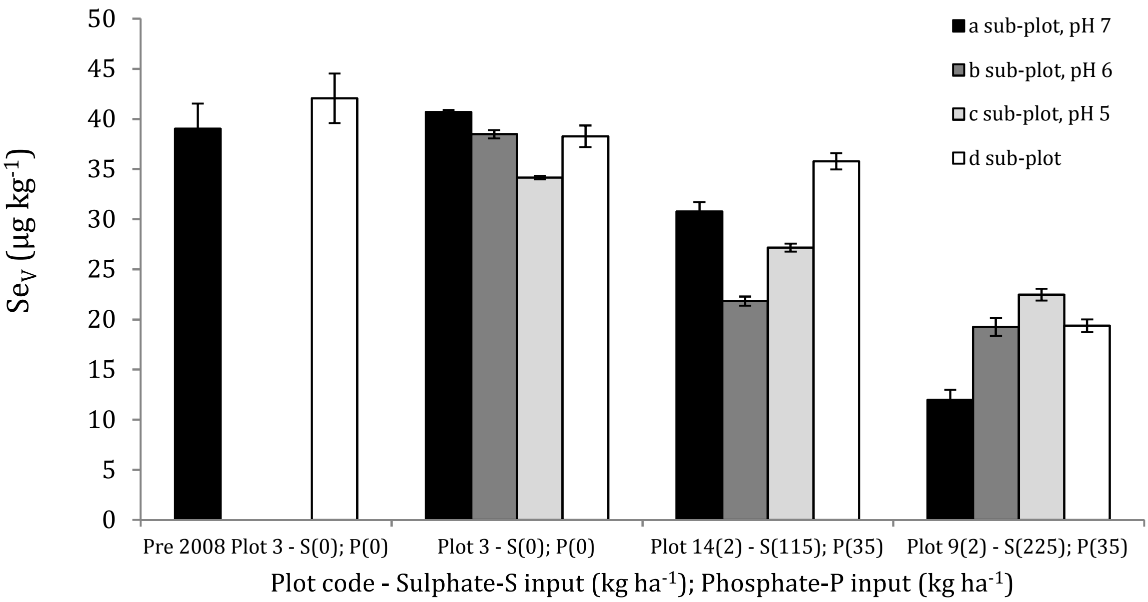


Figure 4: Relationship between inputs of sulphate (S) and phosphate (P) fertilisers and herbage concentrations of (a) iodine and (b) selenium in samples from Plot 3 (control), pre 2008; Plot 3 (control), 2008; Plot 14(2), 2008 and Plot 9(2), 2008 subplots. Error bars show the standard error of 3 replicate analyses.

Table 1: Fertiliser applications and treatment codes defined in the Guide to the Classicals and other Long-term Experiments (Anon, (2006) and in Warren and Johnston (1964).

Plot	Treatment codes	Treatment description	Elemental composition (kg ha^{-1} per treatment)
3	None	None	None
9/2	N2	Ammonium sulphate	N (96); S (110)
	P	Triple superphosphate	P (35)
	K	Potassium sulphate	K (225); S (92)
	Na	Sodium sulphate	Na (15);S (10)
	Mg	Magnesium sulphate	Mg (10); S (13)
12	None	None	None
13/1	FYM/Fishmeal	35,000 kg ha^{-1} FYM every 4 years (1905-1993)	N (240), P (45), K (350), Na (25), Mg (25), S(40), Ca (135)
		Fishmeal applied 2 years after FYM, (until 1995)	
		1907,1910,1915	N (63)
		1959 - 1995, application every 4th year	N (63)
13/2	FYM/pelleted poultry manure	35,000 kg ha^{-1} FYM every 4 years (1905 onwards)	N (240); P (45); K (350); Na (25); Mg (25); S(40); Ca (135)
		Fishmeal applied 2 years after FYM, until 1999.	
		1907,1910,1915	N (63)
		1919 - 1999, application every 4th year	N (63)N (65)
		Pelleted poultry manure every 4 years (from 2003), replacing fishmeal	
14/2	N*2	Sodium nitrate	N (96); Na (157)
	P	Triple superphosphate	P (35)
	K	Potassium sulphate	K (225); S (92)
	Na	Sodium sulphate	Na (15); S (10)
	Mg	Magnesium sulphate	Mg (10) ;S (13)

Table 2: Summary of archived soil and vegetation (cut 1) samples used (*).

Year	Plot	Liming treatment[$]	Soil sample	Vegetation sample
1870	3	U	*	*
1876	3	U	*	*
1876	9, 12, 13, 14	U	*	
1886	3	U	*	*
1904	3	L		*
1904	3	U	*	*
1923	3	L, U	*	*
1939	3	L, U	*	*
1959	3	L	*	*
1959	3	U		*
1971	3	a	*	*
1971	3	d		*
1991	3	a, d	*	*
1998	3	a, d	*	*
2002	3	a, d	*	*
2005	3	a, d	*	*
2008	3	a, b, c, d	*	*
2008	9/2	a, b, c, d	*	*
2008	12	d	*	
2008	13/1	a, b, c, d	*	*
2008	13/2	a, b, c, d	*	*
2008	14/2	a, b, c, d	*	*

[$] L = limed and U = unlimed (pre-1965); a = pH 7, b = pH 6, c = nominal pH 5, d = unlimed (post-1965)

Table 3: Estimated mean annual I and Se inputs from chalk and fertilisers for selected plots compared to estimated total I and Se in soil (assuming 2500 t ha^{-1} of topsoil) and herbage offtake in 2008. Sub-plot refers to liming treatment: a = pH 7, b = pH 6, c = nominal pH 5, d = unlimed. Values were calculated from application rates of chalk and fertiliser. Some lime was added to plots before liming treatments started hence sub-plot 'd' (unlimed) does have some historical lime input. Values are given to three significant figures.

Plot	Sub-plot	Chalk 1870 - 2009 (g ha^{-1} yr^{-1})		Fertiliser 1870 - 2009 (g ha^{-1} yr^{-1})		Combined input* 1870 - 2009 (g ha^{-1} yr^{-1})		Total in soil 2008 (g ha^{-1})		Offtake 2008 (g ha^{-1} yr^{-1})	
		I	*Se*	*I*	*Se*	*I*	*Se*	*I*	*Se*	*I*	*Se*
3	a	6.03	0.036	0.00	0.00	20.0	0.969	12,700	1,240	0.595	0.1173
	b	4.75	0.029	0.00	0.00	18.8	0.961	15,700	1,270	0.569	0.1238
	c	0.59	0.004	0.00	0.00	14.6	0.937	15,400	1,320	0.290	0.0434
	d	0.47	0.003	0.00	0.00	14.5	0.936	16,100	1,310	0.347	0.0612
9/2	a	8.75	0.053	2.24	0.546	25.0	1.53	11,400	1,250	0.844	0.0853
	b	6.71	0.040	2.24	0.546	23.0	1.52	13,000	1,370	0.770	0.1326
	c	2.90	0.017	2.24	0.546	19.1	1.5	11,800	1,380	0.750	0.1391
	d	0.47	0.003	2.24	0.546	16.7	1.48	12,200	1,510	0.567	0.0869
13/1	a	6.00	0.036	4.79	1.26	24.8	1.92	12,300	1,600	0.754	0.1295
	b	4.53	0.027	4.79	1.26	23.3	1.91	12,900	1,260	0.765	0.1041
	c	0.79	0.005	4.79	1.26	19.6	1.89	11,000	1,170	0.334	0.0486
	d	0.47	0.003	4.79	1.26	19.3	1.89	11,700	1,110	0.296	0.0308
13/2	a	6.03	0.036	5.06	0.973	25.1	3.04	11,300	1,130	0.581	0.0833
	b	4.44	0.027	5.06	0.973	23.5	3.03	11,900	1,140	0.586	0.1045
	c	0.73	0.004	5.06	0.973	19.8	3.01	10,700	1,120	0.604	0.0816
	d	0.47	0.003	5.06	0.973	19.5	3.00	11,600	1,120	0.558	0.0874
14/2	a	4.52	0.027	4.42	0.910	22.9	1.87	17,900	1,370	1.38	0.2829
	b	3.39	0.020	4.42	0.910	21.8	1.86	19,500	1,390	1.12	0.1548
	c	0.26	0.002	4.42	0.910	18.7	1.85	17,500	1,310	1.00	0.1789
	d	0.26	0.002	4.42	0.910	18.7	1.85	16,300	1,230	1.19	0.2163

* Combined input from chalk, fertilizer and aerial deposition.

Table 4: Summary of correlations. Values where p > 0.05 are not significant (n.s.).

All plots	**pH**	**SOC**	**I_S**	**I_V**	**I_{OFF}**	**I_{TF}**	**Se_{S-HF}**	**Se_{S-TMAH}**	**Se_{V-HNO3}**	**Se_{V-TMAH}**	**Se_{OFF}**	**Se_{TF}**	**Yield**	**GSR**	**Sulphate**
SOC	n.s.														
I_S	n.s.	n.s.													
I_V	n.s.	n.s.	0.464												
I_{OFF}	n.s.	n.s.	0.387	n.s.											
I_{TF}	n.s.	n.s.	n.s.	0.818	n.s.										
Se_{S-HF}	n.s.	0.375	n.s.	n.s.	0.513	n.s.									
Se_{S-TMAH}	-0.45	0.454	n.s.	n.s.	n.s.	n.s.	0.568								
Se_{V-HNO3}	n.s.	n.s.	0.384	0.72	n.s.	0.569	n.s.	-0.338							
Se_{V-TMAH}	n.s.	n.s.	0.321	0.719	-0.318	0.603	-0.429	-0.411	0.935						
Se_{OFF}	n.s.	n.s.	0.417	n.s.	0.846	n.s.	0.383	n.s.	n.s.	n.s.					
Se_{TF}	n.s.	n.s.	n.s.	0.674	-0.363	0.555	-0.518	-0.419	0.979	0.929	n.s.				
Yield	n.s.	n.s.	n.s.	-0.425	0.884	-0.598	0.549	n.s.	-0.547	-0.61	0.735	-0.621			
GSR	n.s.	n.s.	n.s.	n.s.	0.57	n.s.	0.432	n.s.	-0.462	-0.393	0.304	-0.621	0.541		
Sulphate input	n.s.	0.44	n.s.	-0.509	0.518	-0.565	0.524	0.585	-0.609	-0.705	0.371	-0.638	0.761	0.393	
Phosphate input	n.s.	n.s.	-0.363	-0.58	0.399	-0.482	n.s.	n.s.	-0.799	-0.795	n.s.	-0.792	0.624	0.527	0.638
Control (Plot 3)	**pH**	**SOC**	**I_S**	**I_V**	**I_{OFF}**	**I_{TF}**	**Se_{S-HF}**	**Se_{S-TMAH}**	**Se_{V-HNO3}**	**Se_{V-TMAH}**	**Se_{OFF}**	**Se_{TF}**	**Yield**	**GSR**	**Sulphate**
SOC	n.s.														
I_S	-0.74	n.s.													
I_V	-0.47	n.s.	n.s.												
I_{OFF}	n.s.	n.s.	n.s.	0.436											
I_{TF}	n.s.	n.s.	n.s.	0.835	n.s.										
Se_{S-HF}	n.s.	n.s.	n.s.	n.s.	0.467	n.s.									
Se_{S-TMAH}	n.s.	n.s.	0.685	n.s.	n.s.	n.s.	0.596								
Se_{V-HNO3}	n.s.	n.s.	n.s.	0.476	n.s.	n.s.	-0.555	n.s.							
Se_{V-TMAH}	n.s.	n.s.	n.s.	0.464	n.s.	n.s.	-0.457	n.s.	0.85						
Se_{OFF}	n.s.	n.s.	n.s.	n.s.	0.695	n.s.	n.s.	n.s.	n.s.	n.s.					

Se_{TF}	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.749	-0.517	0.964	0.793	n.s.			
Yield	n.s.	n.s.	n.s.	n.s.	0.864	n.s.	0.482	n.s.	n.s.	n.s.	0.617	-0.453		
GSR	n.s.	n.s.	n.s.	0.55	0.661	n.s.	0.591	n.s.	n.s.	n.s.	n.s.	-0.449	0.524	

2008 Treatments	**pH**	**SOC**	**I_S**	**I_V**	**I_{OFF}**	**I_{TF}**	**Se_{S-HF}**	**Se_{S-TMAH}**	**Se_{V-HNO3}**	**Se_{V-TMAH}**	**Se_{OFF}**	**Se_{TF}**	**Yield**	**GSR**	**Sulphate**
SOC	n.s.														
I_S	n.s.	n.s.													
I_V	n.s.	n.s.	0.474												
I_{OFF}	0.55	n.s.	0.624	n.s.											
I_{TF}	n.s.	n.s.	n.s.	0.66	-0.54										
Se_{S-HF}	n.s.	0.486	n.s.	n.s.	n.s.	n.s.									
Se_{S-TMAH}	-0.66	0.635	n.s.	n.s.	n.s.	n.s.	0.493								
Se_{V-HNO3}	n.s.	n.s.	0.543	0.826	n.s.	n.s.	n.s.	n.s.							
Se_{V-TMAH}	n.s.	n.s.	n.s.	0.848	n.s.	0.562	n.s.	n.s.	0.886						
Se_{OFF}	n.s.	n.s.	0.605	n.s.	0.937	-0.544	n.s.	n.s.	n.s.	n.s.					
Se_{TF}	n.s.	n.s.	0.452	0.818	n.s.	0.472	n.s.	n.s.	0.965	0.903	n.s.				
Yield	0.49	n.s.	n.s.	n.s.	0.894	-0.774	0.33	0.108	-0.185	-0.421	0.834	-0.277			
GSR	*	*	*	*	*	*	*	*	*	*	*	*	*		
Sulphate input	n.s.	0.577	n.s.	-0.578	0.447	-0.653	n.s.	0.604	-0.461	-0.699	n.s.	-0.568	0.695	*	
Phosphate input	n.s.	n.s.	n.s.	-0.728	n.s.	n.s.	n.s.	n.s.	-0.791	-0.813	n.s.	-0.76	n.s.	*	n.s.