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Long-term organic matter application reduces cadmium but not zinc concentrations in wheat

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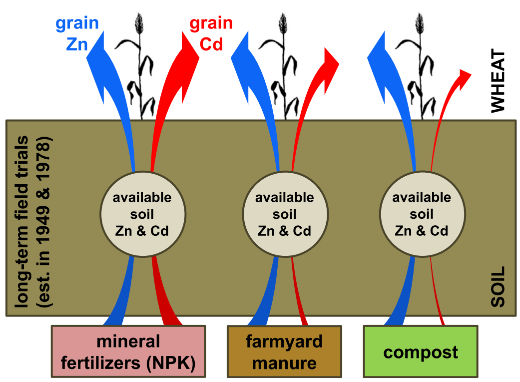
HIGHLIGHTS

- Impact of fertilization on Zn and Cd in wheat was studied in long-term field trials.
- DGT and DTPA were used to measure plant-available soil metals.
- DGT-available soil Cd positively correlated with wheat shoot and grain Cd.
- Wheat grain Cd but not Zn was reduced by long-term organic fertilization.

GRAPHICAL ABSTRACT



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ABSTRACT

Wheat is a staple food crop and a major source of both the essential micronutrient zinc (Zn) and the toxic heavy metal cadmium (Cd) for humans. Since Zn and Cd are chemically similar, increasing Zn concentrations in wheat grains (biofortification), while preventing Cd accumulation, is an agronomic challenge. We used two Swiss agricultural long-term field trials, the "Dynamic-Organic-Conventional System Comparison Trial" (DOK) and the "Zurich Organic Fertilization Experiment" (ZOFE), to investigate the impact of long-term organic, mineral and combined fertilizer inputs on total and phytoavailable concentrations of soil Zn and Cd and their accumulation in winter wheat (*Triticum aestivum* L.). "Diffusive gradients in thin films" (DGT) and diethylene-triamine-pentaacetic acid (DTPA) extraction were used as proxies for plant available soil metals. Compared to unfertilized controls, long-term organic fertilization with composted manure or green waste compost led to higher soil organic carbon, cation exchange capacity and pH, while DGT-available Zn and Cd concentrations were reduced.

Abbreviations: BIODYN, organic cropping system of the DOK trial; COM, compost treatment of the ZOFE trial; CONFYM, conventional cropping system (organic and mineral fertilizers) of the DOK trial; CONMIN, conventional cropping system (mineral fertilizers only) of the DOK trial; DGT, diffusive gradients in thin films; DOK, long-term field trial "Dynamic-Organic-Conventional"; DTPA, diethylene-triamine-pentaacetic acid; FYM, farmyard manure treatment of the ZOFE trial; NOFERT, unfertilized control of the DOK trial; NON, unfertilized control of the ZOFE trial; NPK, mineral fertilization treatment of the ZOFE trial; POST, soil sampling after wheat cultivation; PRE, soil sampling before wheat cultivation; ZOFE, long-term field trial "Zurich Organic Fertilizer Experiment".

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The DGT method was a strong predictor of shoot and grain Cd, but not Zn concentrations. Shoot and grain Zn concentrations correlated with DTPA-extractable and total soil Zn concentrations in the ZOFE, but not the DOK trial. Long-term compost fertilization led to lower accumulation of Cd in wheat grains, but did not affect grain Zn. Therefore, Zn/Cd ratios in the grains increased. High Zn and Cd inputs with organic fertilizers and high Cd inputs with phosphate fertilizers led to positive Zn and Cd mass balances when taking into account atmospheric deposition and fertilizer inputs. On the other hand, mineral fertilization led to the depletion of soil Zn due to higher yields and thus higher Zn exports than under organic management. The study supports the use of organic fertilizers for reducing Cd concentrations of wheat grains in the long-term, given that the quality of the fertilizers is guaranteed.

1. Introduction

Zinc (Zn) is an essential micronutrient for all living organisms. However, about one sixth of the human population is affected by insufficient dietary Zn intake (Wessells and Brown, 2012). On the other hand, cadmium (Cd), which is a sister element of Zn with similar chemical behaviour, has no known biological functions and is toxic to humans at comparatively low doses (Nordberg, 2009). Food is the main source of Cd intake in the non-smoking human population (Järup and Akesson, 2009). Wheat as a staple food crop is a major contributor of both Zn and Cd to human diets. Variations in the concentrations of Zn and Cd in wheat grains can make a large difference to their intake by humans. The concentrations of plant available soil Zn and Cd are important determinants for their uptake by crops (Grüter et al., 2017). This means that Zn and Cd uptake not only depends on their total concentrations in soil, but also on other soil factors such as soil pH, organic carbon content (C_{org}), clay content and cation exchange capacity (CEC) (Alloway, 2013; Viala et al., 2017).

Zinc and Cd enter agricultural soils mainly via atmospheric deposition, sewage sludge and organic and mineral fertilizers (Alloway, 2013; Nicholson et al., 2003). Results from different long-term field trials have shown accumulation of Zn (Benke et al., 2008) or Cd (Wu et al., 2012) in soil due to repeated application of farmyard manure. However, phosphate fertilizers applied to agricultural land also represent a major source of metals, primarily Cd (Nziguheba and Smolders, 2008). Agricultural farming practices also alter soil properties that influence the phytoavailability of soil Zn and Cd. Long-term agricultural field trials offer the opportunity to study these effects over entire crop rotations, balancing out some of the seasonal and short-term variations. Investigating various organic and mineral amendments in the long-term, Lair et al. (2006) demonstrated that soil pH was the main factor controlling the behaviour of Zn and Cd in soil and that higher soil pH and C_{org} led to higher adsorption. Various studies showed that diethylene-triamine-pentaacetic acid (DTPA) extractable Zn and Cd concentrations increased with long-term farmyard manure (Lipoth and Schoenau, 2007; Wu et al., 2012) or compost application (Li et al., 2007). This was due to metal inputs, but also the formation of soluble metal-organic complexes and soil pH decrease with farmyard manure or compost. Li et al. (2007) and Wu et al. (2012) showed that long-term inorganic fertilization did not affect DTPA-extractable Zn and Cd compared to organic amendments. In contrast, Grant et al. (2013) found that the application of Cd-rich phosphate fertilizer increased available soil Cd concentrations, and Lorenz et al. (1994) reported that excess application of fertilizer ammonium (NH_4^+) and potassium (K^+) increased Zn and Cd in soil solutions, probably due to a pH decrease and ion exchange mechanisms. More recently, the "diffusive gradients in thin films" (DGT) method was used to assess Zn and Cd availability in agricultural soils (Muhammad et al., 2012; Tandy et al., 2011). DGT is a passive sampling method using a resin to mimic metal absorption by a plant root and has been found to provide a better proxy of soil metal phytoavailability than conventional chemical extraction methods.

Given the large variation in the effect of soil factors on Zn and Cd supply, it is not surprising that the literature also reports a large variability in the effects that farming practices can have on Zn and Cd accumulation in wheat grains. The application of farmyard manure or compost increased grain Zn (Hamner and Kirchmann, 2015; Lipoth and Schoenau, 2007; Wang et al., 2016) or did not significantly affect it (Li et al., 2007; Tlustoš et al., 2016). Similarly, grain Cd was not affected in some studies (Hamner and Kirchmann, 2015; Lipoth and Schoenau, 2007) while Jones and Johnston (1989) found a decrease in grain Cd over time in farmyard manure treated plots, which they explained with increased Cd retention due to soil organic matter accumulation. In contrast to organic fertilizer application, Wangstrand et al. (2007) found increased grain Cd with increasing nitrogen (N) fertilization rate and explained this with acidification effects or ion exchange between added calcium (Ca^{2+}) and Cd^{2+}. Nitrogen fertilization is also known to be beneficial for grain Zn accumulation which is attributed to the role of N-containing compounds in root uptake, root-to-shoot translocation and remobilization of Zn in wheat (Erenoglu et al., 2011). However, by increasing biomass production more than metal uptake, fertilization can also lead to lower metal concentrations in crops. Export of extracted metals with harvested crops can even lead to the depletion of trace elements in agricultural soils over time without replenishment by fertilization (Hamner and Kirchmann, 2015).

Because of these management effects on micronutrient and trace metal phytoavailability, wheat grains are expected to accumulate different amounts of Zn and Cd under organic and conventional farming. While Cd concentrations in grains were generally lower in organic compared to conventional farming (Cooper et al., 2011; Vrcek et al., 2014; Zaccone et al., 2010), the effects on grain Zn were rather inconsistent. Zinc concentrations were either higher in organic than conventional farming systems (Helfenstein et al., 2016; Vrcek et al., 2014; Zaccone et al., 2010), not affected by the farming system (Mäder et al., 2007) or were even lower (Cooper et al., 2011). The differences in grain Cd were attributed to increased Cd retention in soil under organic farming and to the use of Cd-rich mineral P fertilizers in conventional farming (Cooper et al., 2011; Zaccone et al., 2010). On the other hand, higher inputs with manure application in organic farming (Zaccone et al., 2010) and negative effects of mineral phosphorus fertilization on arbuscular mycorrhizal fungi in conventional farming (Ryan et al., 2004) were given as reasons for higher grain Zn accumulation in organic farming systems. Wheat yields are generally higher in conventional compared to organic farming (Mayer et al., 2015) which could also explain lower Zn and Cd concentrations as a result of "dilution" in a larger biomass.

When comparing Zn and Cd uptake and their allocation in plants, it should also be considered that Zn is an essential micronutrient and phytotoxic only at rather high concentrations in plants of about 100–500 mg kg^{-1}, whereas Cd becomes toxic already at comparatively low concentrations of 10–20 mg kg^{-1} (Kabata-Pendias, 2010). While Zn homeostasis includes controlled accumulation in seeds (Sinclair and Krämer, 2012), in general transfer of Cd from the roots to the shoots and from leaves to the seeds is low (Clemens et al., 2013a). There is also evidence for direct competitive interactions between Zn and Cd in uptake and transport in wheat (Hart et al., 2002).

The diversity of effects that farming practices were found to have on Zn and Cd accumulation in wheat demonstrates that only long-term field trials can show how a certain combination of climate, soil and management factors plays out under real-world conditions. Thus, taking the opportunity provided by two well-established long-term field trials with different fertilization strategies in Switzerland (37 and 65 years old), we aimed to assess the potential of various organic matter management schemes to increase Zn concentrations and to reduce Cd accumulation in wheat grains. Comparing these schemes with mineral fertilizer and no fertilization treatments, the specific objectives were

i. to simultaneously investigate both Zn and Cd phytoavailability,
ii. to evaluate DGT and DTPA extraction for assessing long-term management effects on available soil Zn and Cd,
iii. to compare the impact of organic and conventional management on wheat grain Zn and Cd accumulation, and
iv. to establish soil Zn and Cd mass balances for the studied wheat cultures based on an assessment of the major inputs and outputs.

2. Material and methods

2.1. Experimental designs

The two Swiss long-term field trials used for this study were the "Dynamic-Organic-Conventional System Comparison Trial" (DOK) and the "Zurich Organic Fertilization Experiment" (ZOFE). The DOK trial was established in 1978 at Therwil (47°30′9″N, 7°32′22″E, 307 m asl), in north-western Switzerland. It compares two organic and two conventional cropping systems with different fertilization intensities, which are all replicated in 4 randomized blocks (Mäder et al., 2002). All blocks are managed in the same 7-year crop rotation scheme (Table 1). The mean precipitation was 861 mm per year from 2005 to 2015 and the annual mean temperature 11 °C (Source: MeteoSwiss). The soil was classified as a Haplic Luvisol and its texture as a silt loam (IUSS Working Group WRB, 2014). The pH(H_2O) of the plough layer was 6.3 in 1978. Here, we used the unfertilized control (NOFERT) plots, the conventionally managed plots with mineral fertilization only (CONMIN), the conventionally managed plots with combined application of organic (stacked farmyard manure and slurry) and mineral fertilization (CONFYM) and the organically managed plots fertilized with aerobically composted farmyard manure and slurry (BIODYN).

The winter wheat culture of the DOK trial used for this study was established in October 2014 and harvested in July 2015, 256 days after sowing. After mulching the stubble of the previous soya crop, the soil was ploughed 20 cm deep and harrowed for seedbed preparation. Then winter wheat (*Triticum aestivum* L., cv. Wiwa) was sown at a density of 425 grains m^{-2}. The CONMIN plots received 30 kg P ha^{-1} ($Ca(H_2PO_4)_2$) and 105 kg K ha^{-1} (KCl) 22 days before wheat sowing. The CONFYM plots received the same mineral P and K fertilizers at rates of 26 kg P ha^{-1} and 33 kg K ha^{-1}, respectively. Mineral nitrogen was applied in the CONMIN treatment in three doses of 50, 30 and 40 kg N ha^{-1} (NH_4NO_3) at wheat tillering, stem elongation and flag leaf emergence, respectively. CONFYM plots received 55, 30 and 40 kg N ha^{-1}, respectively. No farmyard manure and slurry is applied to winter wheat in the CONFYM system, even though it is regularly applied to maize, potato and grass-clover in the rotation. Composted cattle farmyard manure was applied to the BIODYN plots at a rate of 15 t fresh weight ha^{-1} 21 days before wheat sowing (with a nutrient input of 49.5 kg P ha^{-1}, 143 kg K ha^{-1}, 86 kg N ha^{-1}). Additionally, slurry was applied in the BIODYN treatment split in three doses: 40, 30 and 30 m^3 ha^{-1} at wheat tillering, stem elongation and flag leaf emergence, respectively (with a total nutrient input of 21 kg P ha^{-1}, 238 kg K ha^{-1}, 128 kg N ha^{-1}). In the two conventional cropping systems, herbicides and fungicides were applied, while weeds were controlled mechanically on the BIODYN plots. Furthermore, biodynamic preparations as described in Zaller and Köpke (2004) were applied in homoeopathic doses in BIODYN.

The ZOFE trial was established in 1949 at Zurich-Reckenholz (47°25′37″N, 8°31′6″E, 440 m asl), Switzerland, to compare different fertilization regimes. It includes 12 treatments of different organic, inorganic and combined fertilization, which are all replicated in 5 blocks on the same field (Oberholzer et al., 2014). The whole field is managed conventionally in an 8-year crop rotation scheme (Table 1). The mean precipitation was 1017 mm per year from 2004 to 2014 and the annual mean temperature 9.9 °C (Source: MeteoSwiss). The soil was classified as a Luvisol and its texture as a sandy loam (IUSS Working Group WRB, 2014). The pH(H_2O) of the plough layer was 6.5 in 1949. For the study here, we used the unfertilized control (NON) plots and the plots with mineral fertilizer (NPK), stacked cattle farmyard manure (FYM) and green waste compost (COM) application.

The winter wheat culture of the ZOFE trial used for our study was established in November 2013 after a maize crop and harvested in July 2014, 248 days after sowing. After the soil was ploughed 20 cm deep and harrowed for seedbed preparation, winter wheat (*Triticum aestivum* L., cv. CH Claro) was sown at a density of 420 grains m^{-2}. NPK plots were fertilized 28 days before wheat sowing with 26 kg P ha^{-1} (superphosphate) and 66 kg K ha^{-1} (K_2SO_4). Nitrogen was applied in three doses of 60, 50 and 40 kg N ha^{-1} (NH_4NO_3) at wheat tillering, stem elongation and flag leaf emergence, respectively. In the FYM treatment, stacked cattle farmyard manure is applied only every second year and was last applied in April 2013 prior to the maize culture (with a nutrient input of 34 kg P ha^{-1}, 232 kg K ha^{-1}, 130 kg N ha^{-1}). Green waste compost was applied at a rate of 2.5 t dry organic matter ha^{-1} to the COM plots 28 days before wheat sowing (with a nutrient input of 14 kg P ha^{-1}, 50 kg K ha^{-1}, 82 kg N ha^{-1}). Herbicides and fungicides were applied to the entire field during the growing period.

Table 1
7-year and 8-year crop rotations of the DOK (2009–2015) and ZOFE (2007–2014) field trials. The wheat cultures studied are highlighted in bold.

Year	DOK	ZOFE
2007		Maize
2008		Spring barley
2009	Potatoes	Grass-clover ley
2010	Winter wheat	Grass-clover ley
2011	Grass-clover ley	Winter wheat + cover crop (oil radish)
2012	Grass-clover ley	Grain maize
2013	Silage maize + cover crop (*Brassica*)	Grain maize
2014	Soybean	**Winter wheat** + cover crop (berseem clover)
2015	**Winter wheat** + cover crop (mixture)	

2.2. Soil and plant sampling

In both trials, soil samples were collected before (PRE) and after (POST) wheat cultivation. Twelve soil cores were taken on a grid from each plot with an Edelman auger (20 cm depth) and mixed, resulting in around 3 kg of fresh bulk soil. The PRE sampling was performed between the harvest of the previous crop and wheat seedbed preparation and the POST sampling shortly after wheat harvest. One part of each sample was kept at 4 °C for the analysis of available soil metals by means of DGT. The other part was dried at 40 °C, sieved to ≤2 mm and subdivided again into two subsamples. One of them was used for texture analysis, while the other was finely ground for the chemical analyses.

At harvest, three 1 m long sections of wheat rows were randomly selected in each plot and all wheat plants cut 1 cm above the ground, dried at 60 °C, weighed and divided into shoots, grains and chaff. The thousand-kernel-weight (TKW) was determined from two sets of 500 grains per sample. Shoot and grain samples were milled for analysis.

2.3. Soil analysis

Soil texture was determined by the sedimentation method (Agroscope, 1996). Soil pH was measured in 0.01 M $CaCl_2$ extracts of ground soil using a soil-to-solution ratio of 1:2.5 (Agroscope, 1996). Effective cation exchange capacity (CEC) was determined by shaking 0.5 g of soil in a solution of 0.1 M $BaCl_2$ (Hendershot et al., 1993) and analysing the extracted Ca, Mg, K, Na, Al, Fe and Mn cations by inductively coupled plasma optical emission spectrometry (ICP-OES, Vista-MPX CCD Simultaneous, Varian Inc., Palo Alto, CA, USA). Total soil C and N concentrations were determined using an NCS analyser (FlashEA 1112 Series, Thermo Fisher Scientific Inc., Waltham, MA USA). Pseudo-total soil Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, S and Zn concentrations were analysed in aqua regia soil digests (Agroscope, 1996) by ICP-OES or ICP-MS (810, Varian Inc., Palo Alto, CA, USA). Soil P was extracted with 0.5 M $NaHCO_3$ (Olsen P) at a pH of 8.5 and analysed by spectrophotometry (Cary 50 UV–Visible Spectrophotometer, Varian Inc., Palo Alto, CA, USA) (Schoenau and O'Halloran, 2008).

For the assessment of metal phytoavailability, ground soil was shaken in extractant solutions containing 5 mM diethylene-triamine-pentaacetic acid (DTPA), 0.01 M $CaCl_2$ and 0.1 M triethanolamine at pH 7.3 at a soil-to-solution ratio of 1:2 for 2 h (Lindsay and Norvell, 1978). The extracts were analysed for Cd, Cu, Fe, Mn and Zn by ICP-OES (5100, Agilent Technologies, Santa Clara, CA, USA).

Available soil Zn and Cd concentrations of non-dried soil were determined by the "diffusive gradients in thin films" (DGT) method, using DGT soil samplers (DGT Research Ltd., Lancaster, UK) with 0.92 mm diffusive layer thickness and a chelex-100 resin gel. For this purpose, samples (equivalent of 60 g dry soil), which had been stored as field-fresh soil at 4 °C and sieved to ≤2 mm, were moistened to 100% water holding capacity and equilibrated for 24 h at 25 °C. Then the DGT devices were deployed for 72 h at 25 °C following the procedure of Tandy et al. (2011). After deployment, the resin was removed and immersed in 1 mL of supra pure 1 M HNO_3 for 24 h. The acid solution was then 5-fold diluted and analysed for Cd, Cu, Mn and Zn concentrations by ICP-MS (810, Varian Inc., Palo Alto, CA, USA). The DGT solutions obtained from the POST sampling of the DOK plots were analysed using an Agilent 7500ce ICP-MS instrument (Agilent Technologies, Santa Clara, CA, USA). The amount of a metal, M, accumulated by the DGT resin was calculated as (Tandy et al., 2011):

$$M = C(V_{acid} + V_{gel})/f_e \quad (1)$$

where C is the metal concentration of the extract, V_{acid} is the volume of the nitric acid extract, V_{gel} is the resin gel volume, and f_e is a factor accounting for incomplete elution. DGT-available Cd, Cu, Mn and Zn (C_{DGT}) were then calculated as:

$$C_{DGT} = M\Delta g/(DAt) \quad (2)$$

where A is the surface area of the diffusive gel exposed to the soil, t is the deployment time, Δg is the diffusive layer thickness and D is the diffusion coefficient of the metal in the diffusive gel.

2.4. Plant analysis

For elemental analysis, 200 mg of shoot sample were digested in 15 mL of 14.3 M HNO_3 at 120 °C for 90 min and with 3 mL of 9.8 M H_2O_2 at 120 °C for another 90 min in a DigiPREP MS digestion system (SCP Science, Quebec, Canada). Grain material (100 mg) was digested in 1 mL of 14.3 M HNO_3 and 2 mL of 9.8 M H_2O_2 for 30 min at a pressure of 40 bar and a temperature of 240 °C in a microwave digestion system (turboWAVE, MLS GmbH, Leutkirch, Germany). The digests were analysed for Ca, Cu, Fe, K, Mg, Mn, P, S and Zn by ICP-OES (5100, Agilent Technologies, Santa Clara, CA, USA) and for Cd by ICP-MS (7500ce, Agilent Technologies, Santa Clara, CA, USA). Total N and C concentrations of plant samples were analysed with the NCS analyser mentioned above. Shoot and grain Zn/Cd ratios were calculated based on a mass basis (mg kg^{-1}).

2.5. Fertilizer analysis

Samples of the fertilizers applied in the two trials in the year of the experiment were digested in the same DigiPREP MS digestion system used for wheat shoots. Five hundred milligrams of mineral fertilizer was digested in 23 mL of nanopure water and 2 mL of 12.1 M HCl at 120 °C for 90 min and analysed by ICP-OES (5100 7500ce, Agilent Technologies, Santa Clara, CA, USA) for Ca, Cd, Cu, Fe, K, Mg, Mn, Na and Zn.

Farmyard manure and compost samples were dried at 105 °C for 24 h and ashed at 500 °C for 4 h while slurry samples were ashed the same way without prior drying. Aliquots of 1 g ash were then digested in a solution of 2 mL of nanopure water and 8 mL of aqua regia (2 mL of 15.7 M HNO_3 and 6 mL of 12.1 M HCl) at 120 °C for 90 min, following the procedure given by Agroscope (1996), and analysed for Cd, Cu, Fe, Mn and Zn by ICP-OES. For the analysis of Ca, K, Mg, Na and P by ICP-OES, organic fertilizer samples were ashed at 600 °C and digested in 6 M HCl. Total N contents of the organic fertilizer samples were determined using the Kjeldahl method (Agroscope, 1996) and ammonium-N by the steam distillation method (Stevenson, 1996). The organic matter contents were determined by loss on ignition at 600 °C for 2.5 h.

2.6. Zn and Cd balances

Mass balances of soil Zn and Cd inputs and outputs were assessed for the wheat cultures of the study season by accounting for inputs with fertilizer application and outputs with the export of the harvested biomass. Chaff biomass was treated as part of the shoot biomass in this assessment, assuming that differences in metal concentrations between chaff and shoot tissues were not relevant for the resulting balances. The Zn and Cd inputs with farmyard manure into the FYM plots of the ZOFE trial were divided by 2 as farmyard manure is applied only every second year in this treatment while farmyard manure inputs were not taken into account for the CONFYM treatment of the DOK trial, because it is not applied to wheat cultures at all in this treatment. Average values for the atmospheric deposition of Zn (119 g ha^{-1} $year^{-1}$) and Cd (0.8 g ha^{-1} $year^{-1}$) were taken from Alloway (2013). For the estimation of Zn and Cd inputs with seeds we assumed a sowing density of 420 grains m^{-2} and average concentrations of 30 mg Zn kg^{-1} and 40 μg Cd kg^{-1}, based on grain Zn and Cd concentrations reported by Alloway (2013).

2.7. Statistical analysis

All statistical analyses and figures were made with the software R version 3.3.2 (R Core Team, 2013). One-way analysis of variance (ANOVA) was performed to analyse treatment effects in the two trials. In the DOK trial, we applied linear mixed effects models using the function lme of the R package nlme (Pinheiro et al., 2016). The fixed effect of the cropping system (SYSTEM with 4 levels: NOFERT, CONMIN, CONFYM, BIODYN) was analysed treating the BLOCK factor as a fixed effect and considering the DOUBLEFIELD factor as a random effect to account for the fact that field plots of the NOFERT and CONMIN systems were not randomly distributed but adjacent to each other in each block:

$$lme(y \sim SYSTEM + BLOCK, random = \sim 1|DOUBLEFIELD, data) \quad (3)$$

For the analysis of the ZOFE trial we used linear models with the 4 levels of NON, NPK, FYM and COM for the variable FERTILIZER:

$$lm(y \sim FERTILIZER, data) \quad (4)$$

No blocking factor was considered in this case, because there was no indication of spatial correlation between the ZOFE plots for any of the investigated variables.

The function glht from the multcomp package was used for Tukey's HSD pairwise comparisons (Hothorn et al., 2008). Data were transformed as necessary to meet the assumption of normally distributed residual errors and random effects. Differences in treatment means with $p \leq 0.05$ were considered significant. In the ZOFE dataset, one measurement of total soil Cu and one of DTPA Zn were excluded from the analysis after being identified as outliers by the Dixon test. Furthermore, Cd concentrations of two grain samples of the ZOFE trial were below the detection limit of the ICP-MS instrument and half the value of the detection limit was used for further calculations.

3. Results

3.1. Soil properties

The two field sites generally differed in several basic soil characteristics that are important for Zn and Cd dynamics. The contents of clay, silt and organic carbon were on average higher in the DOK compared to the ZOFE soil (Table 2). The cation exchange capacity, which is governed by these parameters, and the level of macronutrients were therefore also higher in the DOK trial. However, there was a wider range and a more distinct differentiation of C_{org} and CEC among the treatments of the ZOFE trial. The soil pH of both field trials was moderately acidic and varied in a similar range between around 5 and 6 (Fig. 1).

In both field trials, the different long-term management schemes had strong impacts on chemical soil properties, including pH, organic carbon (C_{org}), cation exchange capacity (CEC) and nutrient element concentrations (Fig. 1, Tables 2 and 3), and particularly on available soil Zn and Cd concentrations (Figs. 2 and 3). Total soil organic carbon and soil pH were highest in the COM treatment and lowest in the unfertilized control (NON) in the ZOFE trial. In the DOK trial, these two parameters were also significantly higher in the treatment with added compost (BIODYN) compared to the NOFERT treatment. Cation exchange capacity was closely correlated with C_{org} in both trials, and in the ZOFE trial also with soil pH, while the correlation between pH and CEC was only weak in the DOK trial (Figs. S.1 and S.2). In contrast, there was a much stronger correlation between clay content and CEC in the DOK trial than in the ZOFE trial (Figs. S.1 and S.2). In both trials, the clay content also showed substantial variation between replicate plots (Table 2). In the DOK trial, the variability in texture did not average out within the treatments like in the ZOFE trial, as the clay content was significantly higher and the sand content significantly lower in the BIODYN than in the CONFYM treatment. This variation in clay between treatments and plots in the DOK trial may also be the reason why there was no significant difference in the C_{org} content and the CEC between the CONFYM and the CONMIN treatment, which would have been expected due to the regular application of organic fertilizers (Table 2).

The total concentrations of soil N showed close correlations with soil organic carbon in both trials (Tables 3 and S.1) and were lower on the unfertilized control plots than on the compost plots (BIODYN & COM) in both trials. Total K concentrations were also lower in the unfertilized control plots than in the plots with mineral fertilization in both trials. Total soil Cd and Zn concentrations were higher in the FYM treatment than in the NPK treatment in the ZOFE trial, while they did not show significant treatment effects in the DOK trial.

In the DOK trial, DTPA-extractable Zn after harvest (POST) was higher in the cropping system with compost fertilizer than in the mineral fertilized and unfertilized control plots (Fig. 2). DTPA-extractable Cd however was very similar in all the treatments. In the ZOFE trial, both DTPA Zn and Cd levels were higher in the farmyard manure than in the mineral fertilized treatments. In contrast to the DOK trial, soil total and DTPA-extractable Zn and Cd concentrations were strongly correlated (Table 4). All DTPA Zn concentrations lay clearly above 0.5 mg kg^{-1}, a threshold below which soils are potentially Zn-deficient (Alloway, 2013).

Even though both field trials had similar levels of total and DTPA-extractable soil Zn and Cd concentrations (Table 3 and Fig. 2), the DGT Zn and Cd levels of the ZOFE soils were more than twice as high as the DOK soils (Fig. 3). DGT-available soil Zn and Cd were strongly influenced by the different long-term treatments. Generally, they were lowest in the treatments using compost (BIODYN and COM). In both field trials, DGT Zn and Cd concentrations showed strong negative correlations with soil C_{org}, pH and CEC (Table 4). In the DOK trial, DGT Zn was also negatively correlated to the clay content while no such relationship could be found in the ZOFE trial.

3.2. Wheat growth and accumulation of Zn and Cd

Wheat grain yields were 5.4 t ha^{-1} in the two conventional cropping system treatments of the DOK trial (Table 5) which were close to average yields of winter wheat (6 t ha^{-1}) in Swiss conventional farming with mineral fertilization (Flisch et al., 2009). The BIODYN treatment produced yield of 5.1 t ha^{-1}, which was above average for organic farming (Rudmann and Willer, 2005). In the ZOFE trial however, wheat yields were very high in the NPK treatment (7.5 t ha^{-1}) while they remained low (ca. 2.5 t ha^{-1}) under organic fertilization. In both trials, higher yields were associated with higher numbers of ears and grains per hectare (Table S.4), as previously shown for the DOK trial (Mayer et al., 2015).

The comparison of nutrient concentrations in shoots and grains among the different treatments shows us the nutrient limitations for biomass production. In the DOK trial, shoot K and grain N concentrations were lowest in the NOFERT treatment (Tables S.3 and S.4). In the ZOFE trial, shoot and grain N and shoot K concentrations were very low in the NON, FYM and COM, compared to the NPK treatment. According to Reuter and Robinson (1986), the grain N concentrations in these three treatments indicate marginal N supply for wheat growth.

Table 2
Soil characteristics measured before (PRE) wheat cultivation. Values shown are means and standard errors of 4 (DOK) and 5 (ZOFE) plot replicates. Means with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple comparisons. Significance letters were assigned separately for each trial.

Field trial	Treatment	Clay (g kg^{-1})	Silt (g kg^{-1})	Sand (g kg^{-1})	C_{org}[a] (g kg^{-1})	CEC (cmol(+) kg^{-1})	Olsen P (mg kg^{-1})
DOK	NOFERT	177 ± 28ab	682 ± 24a	141 ± 43ab	11.7 ± 1.0 b	11.0 ± 1.8ab	5.5 ± 0.7b
	CONMIN	238 ± 30ab	640 ± 40a	122 ± 46ab	13.4 ± 1.2ab	12.9 ± 1.7ab	18.1 ± 1.3a
	CONFYM	141 ± 23b	712 ± 4a	147 ± 24a	13.4 ± 0.3ab	10.2 ± 0.2b	15.7 ± 1.3a
	BIODYN	214 ± 11a	694 ± 25a	92 ± 16b	15.9 ± 1.4a	13.5 ± 1.6a	9.0 ± 0.9b
ZOFE	NON	107 ± 14a	243 ± 9a	650 ± 23a	7.5 ± d	6.0 ± 0.4c	21.6 ± 2.3b
	NPK	130 ± 4a	263 ± 4a	607 ± 8a	8.6 ± c	7.3 ± 0.5bc	33.5 ± 1.8a
	FYM	108 ± 12a	249 ± 10a	643 ± 20a	10.0 ± b	8.0 ± 0.5b	3.8a
	COM	129 ± 7a	258 ± 14a	613 ± 20a	12.1 ± a	11.5 ± 0.1a	3.3ab

[a] Total soil C was considered to be organic C because there was no inorganic C present in all soils.

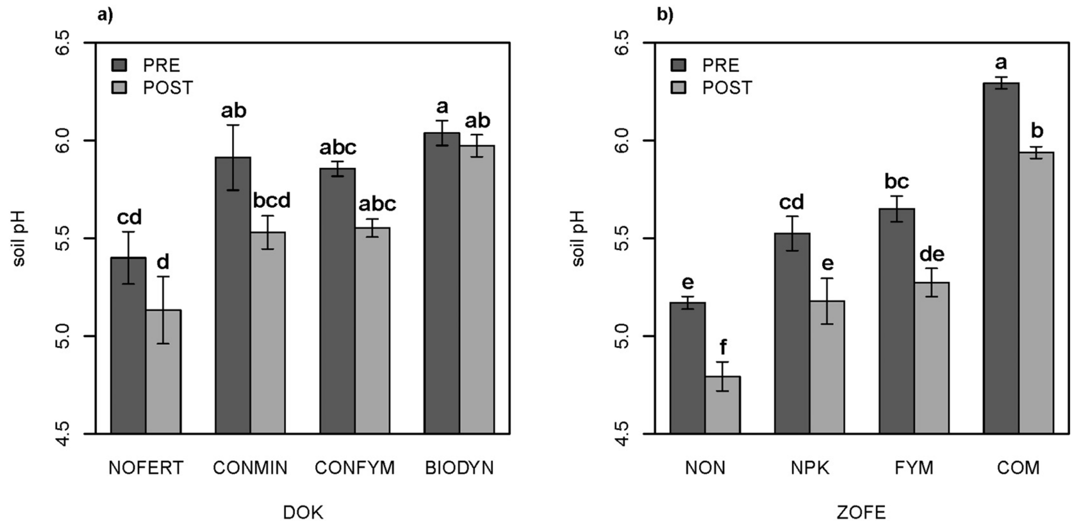

Fig. 1. Soil pH($CaCl_2$) in the DOK (a) and the ZOFE trial (b) before (PRE) and after (POST) wheat cultivation (mean ± SE, n = 4 (DOK) and n = 5 (ZOFE)). Means with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple comparisons.

While grain Zn concentrations were around 30 mg kg^{-1} in both trials, shoot Zn concentrations were about 3 to 5 times lower in the DOK than in the ZOFE trial (Fig. 4). Although shoot Zn was highest, grain Zn was lowest in the unfertilized DOK treatment, while there were no significant differences between the treatments with fertilization. In the ZOFE trial, the only significant treatment effect on plant Zn accumulation was that shoot Zn was much lower in the NPK treatment than in the FYM and in the unfertilized control treatment. Shoot and grain Zn concentrations positively correlated with each other in the ZOFE ($R^2 = 0.44^{**}$), but not in the DOK trial. However, only in the DOK trial, grain Zn correlated with grain N concentrations ($R^2 = 0.52^{**}$).

The overall levels of shoot and grain Cd concentrations in both trials were comparatively low, with average grain Cd levels ≤ 50 μg kg^{-1} (Fig. 5). Shoot Cd concentrations were more than twice as high in the unfertilized control than in the BIODYN treatment in the DOK trial, while the other two treatments were between these extremes. They were also almost two times as high in the NON as in the COM treatment in the ZOFE trial; but here, the other two treatments were not significantly different from the unfertilized control. Grain Cd concentrations showed similar trends as shoot Cd. The correlations between shoot and grain Cd concentrations thus were strong (DOK: $R^2 = 0.57^{***}$; ZOFE: $R^2 = 0.48^{**}$), but there were still substantial variations in grain Cd that was independent of shoot Cd. In particular, grain Cd was not higher in the unfertilized control of the DOK trial than in the NPK and FYM treatments. Furthermore, it is worth to note that Zn/Cd ratios were highest in shoots and grains in the treatments with compost fertilization (Table 5). In the ZOFE trial Zn/Cd ratios in the grains were not significantly different among treatments ($p = 0.09$) but showed the same trend as in the shoots.

Both shoot and grain Cd concentrations were strongly correlated with DGT-available soil Cd concentrations, but not with DTPA-extractable soil Cd (Table 4). In contrast, shoot and grain Zn concentrations were more strongly correlated with DTPA-extractable Zn than with DGT-available soil Zn concentrations in the ZOFE trial, while there was no correlation of shoot and grain Zn with either pool of soil Zn in the DOK trial. In the DOK trial, all fertilized treatments resulted in similar total grain Zn uptake, reflecting the fact that there was little variation in grain Zn concentrations and yields among them (Table 5). However, due to the decreased grain Cd concentrations in the BIODYN treatment, total grain Cd uptake was as low in this treatment as in the unfertilized control, and for the same reason it was as low in the COM as in the unfertilized treatment in the ZOFE trial. Conversely, the high biomass production led to the highest Zn and Cd uptakes in the NPK treatment in the ZOFE trial.

That Cd uptake was reduced by the COM and the BIODYN treatments is also evident in the low values of the soil-to-shoot transfer factors using total or DTPA soil Cd concentrations (Table S.5). This was not the case for Zn. However, when using DGT-available metal concentrations, the BIODYN and the COM treatments' soil-to-shoot transfer factors were highest for both Zn and Cd, due to the very low levels of DGT Zn and Cd in these treatments. The shoot-to-grain transfer factors were >1 for Zn, but <1 for Cd. In the DOK trial, they were lower in the unfertilized treatment than with purely mineral fertilization (CONMIN), while there was no significant difference between the treatments with and without fertilization in the ZOFE trial.

Table 3
Total soil element concentrations measured before (PRE) wheat cultivation. Values shown are means of 4 (DOK) and 5 (ZOFE) plot replicates. Means with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple comparisons. Significance letters were assigned separately for each trial.

Field trial	Treatment	Ca (g kg^{-1})	Cd (mg kg^{-1})	Cu (mg kg^{-1})	Fe (g kg^{-1})	K (g kg^{-1})	Mg (g kg^{-1})	Mn (g kg^{-1})	N (g kg^{-1})	Na (mg kg^{-1})	P (g kg^{-1})	S (g kg^{-1})	Zn (mg kg^{-1})
DOK	NOFERT	2.5ab	0.28a	16b	14a	1.9b	3.7a	0.77a	1.3b	61a	0.59c	0.17b	65a
	CONMIN	2.6ab	0.31a	18ab	14a	2.5a	3.9a	0.77a	1.5ab	78a	0.72a	0.21a	65a
	CONFYM	2.5b	0.31a	20a	12a	2.2ab	3.3a	0.79a	1.4ab	75a	0.66b	0.23a	59a
	BIODYN	2.9a	0.30a	17ab	13a	2.1ab	3.7a	0.72a	1.7a	60a	0.64bc	0.25a	66a
ZOFE	NON	1.3c	0.25ab	20ab	16a	1.2b	3.5a	0.49b	0.8d	75a	0.46b	0.14c	64ab
	NPK	1.7b	0.20b	16c	17a	1.6a	3.6a	0.62a	0.9c	65a	0.59a	0.15bc	54b
	FYM	1.7b	0.31a	22a	17a	1.4a	3.6a	0.49b	1.0b	76a	0.58a	0.17b	75a
	COM	2.4a	0.26ab	18bc	16a	1.4a	3.5a	0.52b	1.1a	75a	0.55a	0.21a	70a

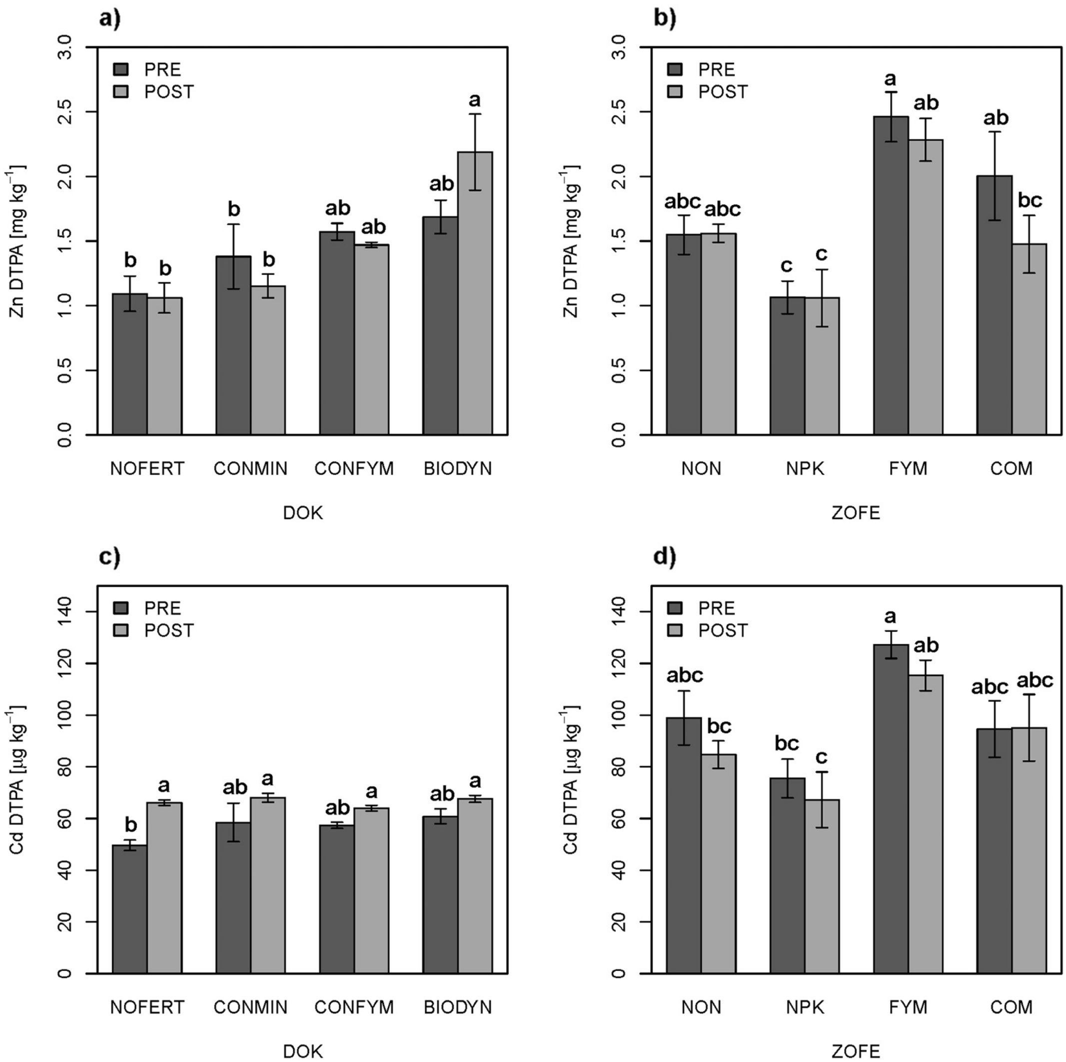

Fig. 2. DTPA-extractable Zn and Cd in the DOK (a, c) and the ZOFE (b, d) trial before (PRE) and after (POST) wheat cultivation (mean ± SE, n = 4 (DOK) and 5 (ZOFE)). Means with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple comparisons.

3.3. Zn and Cd balances

When comparing Zn inputs into soils from fertilizer application and atmospheric deposition to Zn outputs via export of harvested crop for the studied wheat cultivation period, organic fertilizers proved to be the major sources of Zn, exceeding outputs several times and thus leading to positive balances (Table S.6). Organic fertilizer Zn inputs were not accounted for in the CONFYM system of the DOK trial because no organic fertilizers were applied to the wheat cultures. Zn inputs from atmospheric deposition and mineral fertilizers were smaller than for organic fertilizers, but still high with 30 to 120 g ha^{-1} year^{-1}, depending on the type and amount of fertilizer applied. Zn inputs from seeds were negligible. High yields and comparatively low Zn inputs with mineral fertilization led to negative Zn balances in the treatments with only mineral fertilizers during the wheat cultures, which include CONFYM. In the unfertilized control treatments, Zn outputs from harvest approximately balanced the inputs from atmospheric deposition. Thus, the Zn balance was close to zero.

Organic fertilization played a smaller role as inputs for Cd, compared to mineral P fertilizers, and atmospheric deposition (Table S.6) than for Zn. As for Zn, the outputs of Cd from crop harvest were of a similar magnitude to the inputs from mineral fertilizers and deposition. As a result, all balances were positive, but not as excessive as for Zn. The highest input was through a contaminated P fertilizer applied in the ZOFE trial with a concentration of 123 mg Cd kg^{-1} P which was clearly above the maximum permitted concentration in P fertilizers of 50 mg Cd kg^{-1} P set by the Swiss law (ChemRRV, 2005).

4. Discussion

4.1. Soil Zn and Cd availability

Total soil concentrations of Zn and Cd can be explained by the balance of inputs and outputs of the system. Higher total soil Zn concentrations in the FYM and COM treatments compared to the NPK treatment of the ZOFE trial can be explained by the large inputs due to farmyard manure application (Benke et al., 2008; Nicholson et al., 2003) and compost in the FYM and COM treatments and the high exports through wheat harvest in the NPK treatment. The treatment effects on total soil Cd showed similar patterns and suggest that the same explanations can be given for these differences as was shown by Schweizer et al. (2018) for farms using compost. However, this is not explained by the calculated Cd balances for one wheat cultivation period. Here the inputs with mineral P fertilizers played a more important role compared to the inputs from farmyard manure. However, the Cd-rich P fertilizer applied in the ZOFE trial had only been used since 2011 and an accumulation of

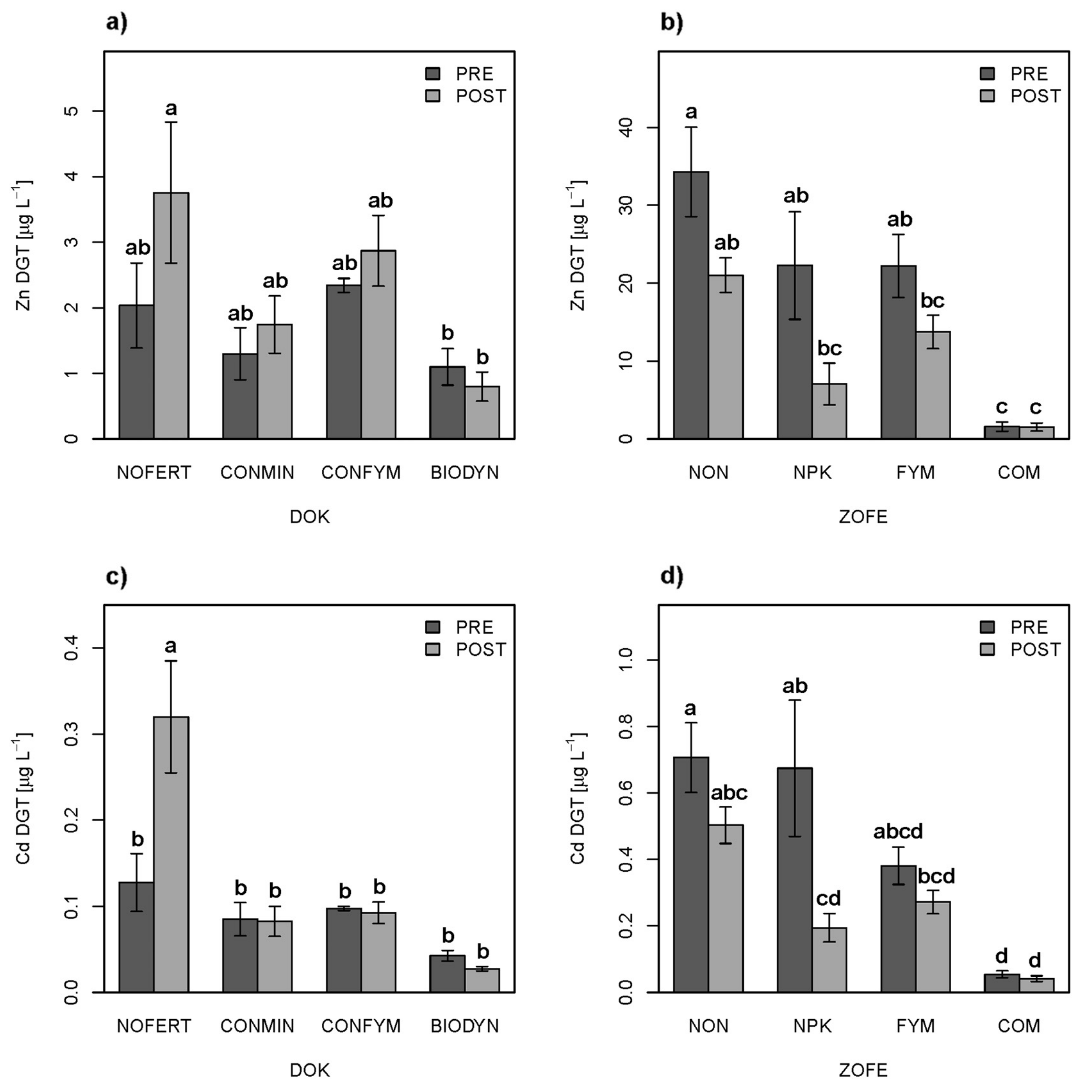

Fig. 3. DGT-available Zn and Cd in the DOK (a, c) and the ZOFE (b, d) trial before (PRE) and after (POST) wheat cultivation (mean ± SE, n = 4 (DOK) and n = 5 (ZOFE)). Means with the same letter are not significantly different at $p \le 0.05$ according to Tukey multiple comparisons.

soil Cd in the NPK treatment could not be measured. The fact that there were no significant differences in total soil Zn and Cd between the organically and chemically fertilized treatments in the DOK trial may be due to the similar yields for all treatments leading to similar Zn and Cd exports and due to the similar C_{org} concentrations for CONFYM and CONMIN leading to similar amounts of Zn and Cd binding to the soil.

The differences in DTPA-extractable Zn and Cd concentrations between the treatments of the ZOFE trial mirrored the pattern of total soil Zn and Cd concentrations. Changes in extractable soil Zn and Cd with long-term manure application are in line with findings in previous field studies (Benke et al., 2008). In the DOK trial however, only DTPA-extractable Zn concentrations were increased in the treatment with composted manure (BIODYN), while Cd DTPA concentrations were not affected by treatment. This lack of distinction between treatments could be due to the same processes mentioned above for total Zn and Cd.

Comparing the results of the two trials suggests that the differences in DGT-available soil Zn between treatments were determined to a

Table 4
Coefficients of determination (R^2) of simple linear regressions between DGT-available and DTPA-extractable soil Zn and Cd and soil and plant parameters. Asterisks represent level of significance (*$p \le 0.05$; **$p \le 0.01$; ***$p \le 0.001$). Negative correlation coefficients are indicated by (−). C_{org}, CEC, clay content, total soil Zn and Cd concentrations were measured before wheat cultivation (PRE, Table 3), while pH($CaCl_2$), DGT-available soil Zn and Cd, DTPA-extractable soil Zn and Cd were measured after wheat cultivation (POST).

	C_{org}		pH($CaCl_2$)		CEC		Clay		Soil Zn or Cd		Zn or Cd shoot		Zn or Cd grain	
	DOK	ZOFE	DOK	ZOFE	DOK	ZOFE	DOK	ZOFE	DOK	ZOFE	DOK	ZOFE	DOK	ZOFE
Zn DGT (log)	(−) 0.80***	(−) 0.57***	(−) 0.40**	(−) 0.69***	(−) 0.69***	(−) 0.70***	(−) 0.30*	(−) 0.04	(−) 0.42**	0.02	0.24	0.20*	(−) 0.14	0.33*
Cd DGT (log)	(−) 0.58***	(−) 0.72***	(−) 0.72***	(−) 0.85***	(−) 0.27*	(−) 0.83***	(−) 0.17	(−) 0.06	(−) 0.03	0.01	0.85***	0.68***	0.60***	0.43**
Zn DTPA	0.04	0.02	0.45**	0.00	(−) 0.00	(−) 0.00	0.00	(−) 0.02	(−) 0.03	0.79***	(−) 0.05	0.73***	0.14	0.46**
Cd DTPA	(−) 0.01	0.11	0.03	0.06	0.01	0.05	0.18	(−) 0.01	0.06	0.88***	(−) 0.00	0.09	(−) 0.01	0.01

Table 5
Wheat yield components, Zn and Cd uptake and ratios at harvest. Values shown are means of 4 (DOK) and 5 (ZOFE) plot replicates. Means with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple comparisons. Significance letters were assigned separately for each trial.

Field trial	Treatment	Chaff biomass ($t\ ha^{-1}$)	Shoot biomass ($t\ ha^{-1}$)	Grain biomass ($t\ ha^{-1}$)	TKW (g)	Grain Zn uptake ($g\ ha^{-1}$)	Grain Cd uptake ($mg\ ha^{-1}$)	Zn/Cd shoot	Zn/Cd grain
DOK	NOFERT	0.9b	3.8b	3.1b	34b	87b	156b	69b	555b
	CONMIN	1.7a	8.0a	5.4a	39a	167a	259a	51b	647b
	CONFYM	1.6a	8.0a	5.4a	39a	172a	226a	90ab	783b
	BIODYN	1.6a	9.1a	5.1a	39a	162a	146b	119a	1113a
ZOFE	NON	0.7c	1.7c	1.6b	37b	51c	65b	135b	790a
	NPK	1.8a	6.4a	7.5a	39ab	204a	203a	90c	1104a
	FYM	0.9b	2.4b	2.8b	40a	93b	67b	170b	2475a
	COM	0.8b	2.1bc	2.4b	39ab	63bc	35b	270a	2543a

similar extent by the variations in soil pH and C_{org}, while DGT-available soil Cd was primarily governed by soil pH, with C_{org} playing a secondary role. Higher soil pH and CEC led to a stronger binding of Zn and Cd as expressed by lower DGT values. These conclusions are in line with results reported by other authors who found the same soil parameters effected Zn and Cd DGT concentrations and metal binding in soils (Lair et al., 2006; Muhammad et al., 2012; Perez and Anderson, 2009; Tian et al., 2008). The findings also agree with the results of a previous subplot experiment performed on the ZOFE trial in which DGT was suitable for monitoring the seasonal dynamics in soil Zn and Cd availability (Grüter et al., 2017).

Comparing DGT-available and DTPA-extractable soil metal concentrations shows that DGT Zn and Cd concentrations were more strongly affected by variations in soil pH and CEC, while DTPA-extractable Zn and Cd concentrations were more closely related to total soil Zn and Cd concentrations. Positive relationships between total and DTPA-

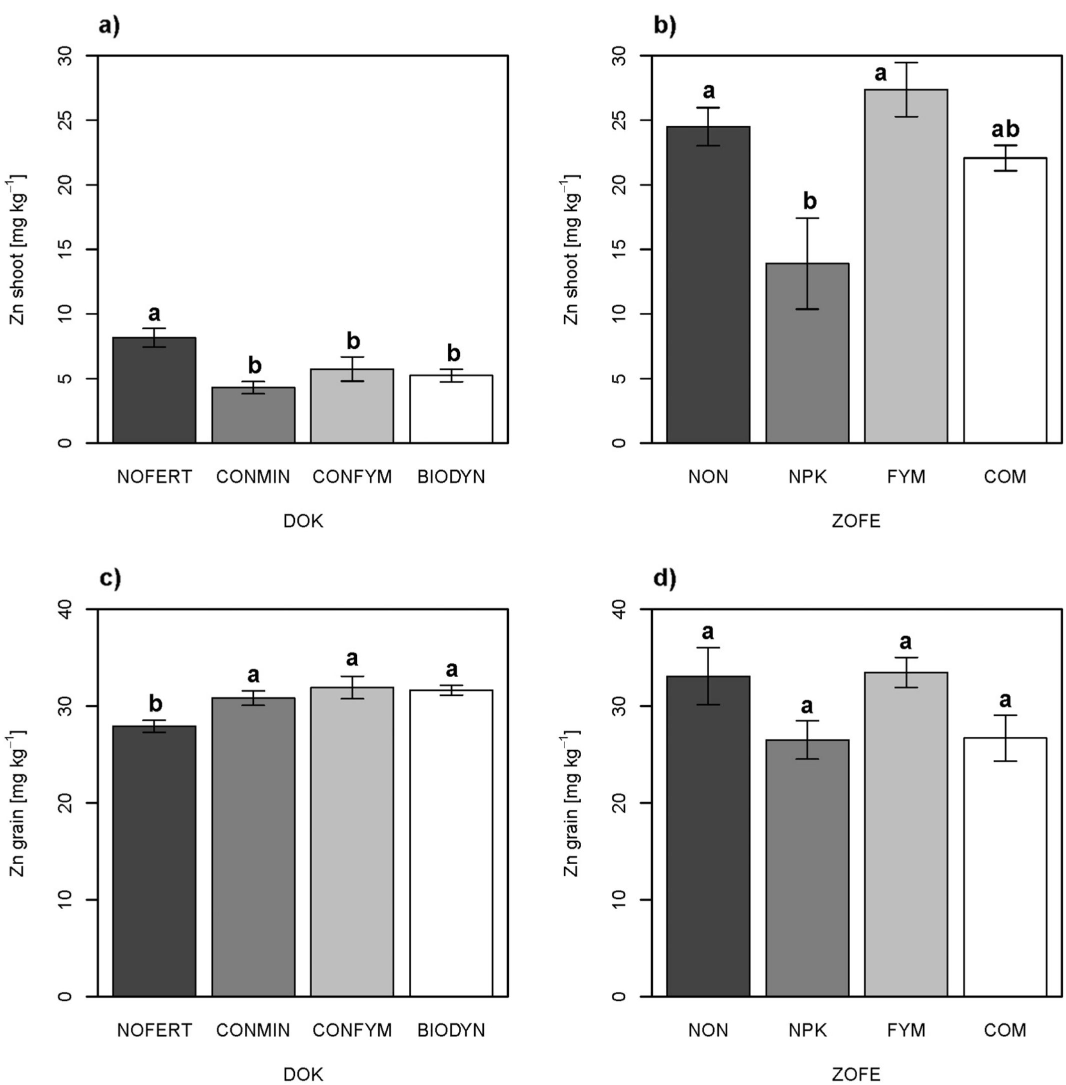

Fig. 4. Zn concentrations in wheat shoots and grains of the DOK (a, c) and ZOFE (b, d) trial (mean ± SE, n = 4 (DOK) and n = 5 (ZOFE)). Means with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple comparisons.

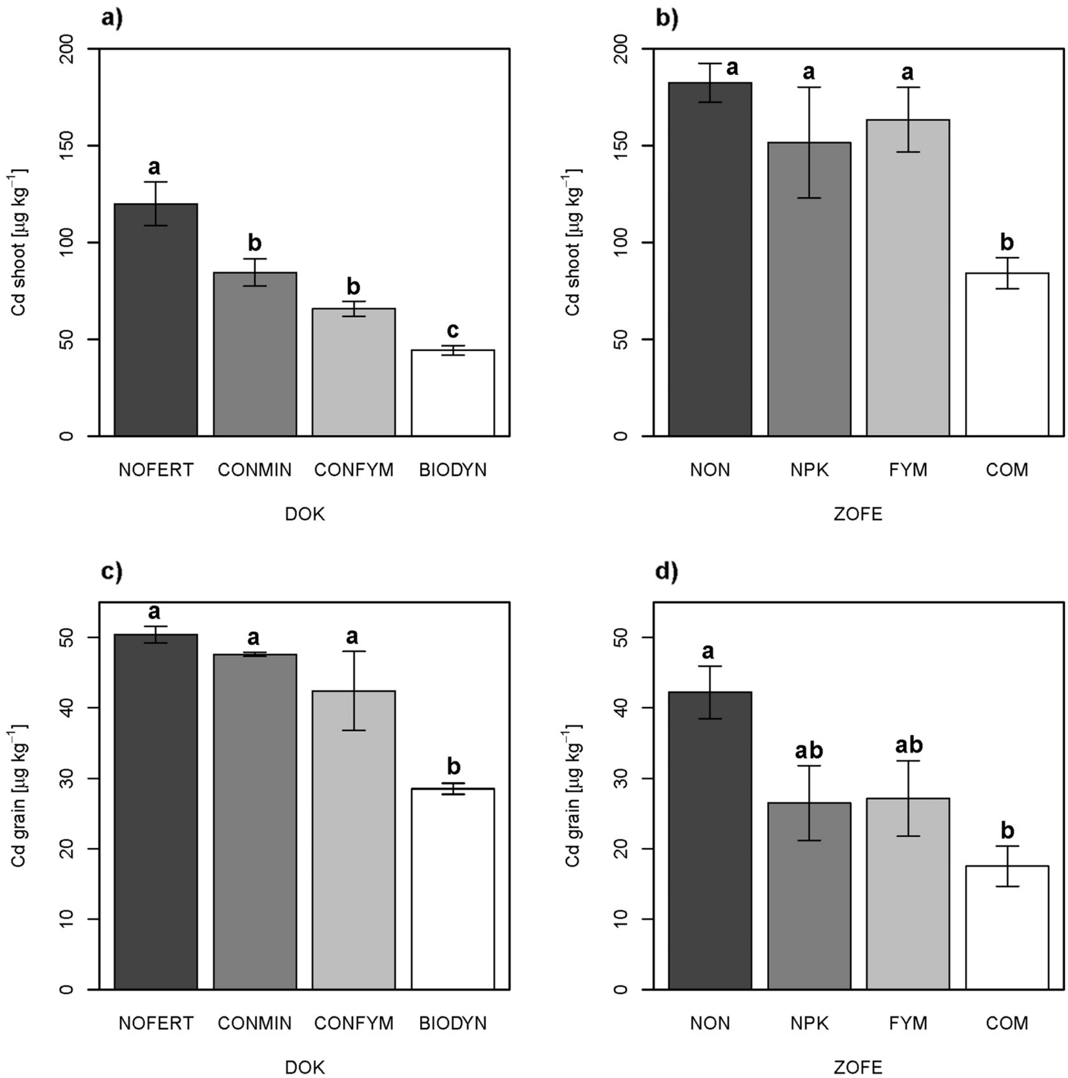

Fig. 5. Cd concentrations in wheat shoots and grains of the DOK (a, c) and ZOFE (b, d) trial (mean ± SE, n = 4 (DOK) and n = 5 (ZOFE)). Means with the same letter are not significantly different at $p \leq 0.05$ according to Tukey multiple comparisons.

extractable soil Zn and Cd concentrations in contrast to DGT-available Zn and Cd confirm previous findings (Grüter et al., 2017; Helfenstein et al., 2016). Grüter et al. (2017) suggested DTPA extracts Zn and Cd more strongly bound to soil particles or soil organic matter, which remains unavailable to the DGT device. This can be attributed to the fact that the DTPA extraction is a pseudo-equilibrium between the extractant and soil while DGT metal uptake is a diffusion limited process responding to kinetics of release from the soil (Tandy et al., 2011).

These differences in long-term management effects on DGT and DTPA soil metal concentrations demonstrate that these two methods cannot be used interchangeably for assessing the same element fraction in the soil matrix. However, both methods and especially their combination are useful to identify differences in the soil chemistry and behaviour of different trace elements. Linking the results of DGT and DTPA analysis with plant element concentrations can therefore help understand differences in plant uptake mechanisms between trace elements.

4.2. Wheat growth and Zn and Cd uptake

Biomass is one factor directly affecting element concentrations in shoot and grain tissues (Liu et al., 2014). While low K shoot concentrations in NOFERT of the DOK indicate limitations for vegetative growth, at grain filling it had the highest grain K concentrations, but the lowest N concentrations. This indicates that insufficient transfer of N limited grain protein production, despite comparatively high shoot N concentrations. The low shoot and grain N concentrations and the low yields in the NON, FYM and COM treatments of the ZOFE trial compared to the NPK treatment indicate that N was the biomass-limiting nutrient here. In contrast to the DOK trial, the FYM and COM treatments had no slurry applied, but only solid organic fertilizer, which is a source of slow N release and explains the low yields due to the missing synchrony between available soil N supply and plant N demand. However, shoot K concentrations were also low, indicating co-limitation by K, which is in line with Oberholzer et al. (2014) who also reported this for the ZOFE trial.

Although DTPA-extractable soil Zn was a better predictor of shoot and grain Zn variation in the treatments of the ZOFE trial than DGT-available soil Zn, it does not explain the very different levels of shoot Zn accumulation between the two trials, in contrast to DGT Zn. It appears that the difference in available soil Zn and Zn uptake by wheat between the two experimental sites was dominated by differences in soil properties that were more adequately reflected by DGT-available than DTPA-extractable Zn, in particular CEC and soil pH. The correlation between DTPA-extractable soil Zn and Zn uptake by wheat in the ZOFE trial confirms findings of our previous experiment using the NPK and FYM treatments of this trial (Grüter et al., 2017). In the

DOK trial however, wheat Zn concentrations were neither correlated to DTPA, nor to DGT Zn. Compared to the other treatments, Zn phytoavailability was overestimated by DTPA Zn in the BIODYN treatment, while it was underestimated by DGT. The roots could probably access a part of the DTPA-extractable Zn pool that was not DGT-available, including organically bound Zn, via mobilization mechanisms such as the exudation of protons, phytosiderophores or organic acid anions (White and Broadley, 2009).

The fact that grain Zn concentrations were higher than shoot Zn concentrations in all treatments of both trials can be attributed to the translocation of Zn during grain-filling from the shoots, in particular from the flag leaves, into the developing grains (Pearson and Rengel, 1994), where it appears to be primarily bound by proteins (Xue et al., 2014). However, the production of N-containing chelating ligands (e.g. nicotianamine) and membrane transporter proteins (ZIP, YSL or HMA families) which are needed for Zn translocation within the plant, as well as grain proteins, rely on adequate N nutrition (Clemens et al., 2013b; Erenoglu et al., 2011). A positive correlation between grain N and Zn concentrations as found in several previous field studies (Xue et al., 2012; Zhao et al., 2009) was only found in the DOK trial however. In the ZOFE trial, grain Zn concentrations were determined rather by the soil Zn levels than by the sink effect of grain proteins, confirming previous findings from the same field trial (Grüter et al., 2017). This may be due to the limited supply of N in most treatments. In the NOFERT system of the DOK trial with limited available N, there was also a poor transfer of Zn between the shoots and the grains. This can be explained by a limited translocation capacity during grain-filling as the initial pool of available N became exhausted. In contrast, translocation was apparently higher in the mineral (CONMIN and NPK) than in the organic fertilizer treatments, probably due to the timing of N application at the beginning of the grain-filling phase, supporting previous findings from a long-term N fertilization field study (Shi et al., 2010).

In all fertilized treatments in the two trials, grain Zn concentrations were slightly below biofortification target levels of 37 mg kg^{-1} (Bouis and Saltzman, 2017). In the ZOFE trial, they tended to be decreased in the NPK treatment, where the largest grain biomass was produced (dilution effect) and total soil Zn was depleted, and in the COM treatment, where DGT Zn was extremely low.

In contrast to DGT Zn, DGT-available Cd was an excellent predictor of shoot and grain Cd concentrations in both field trials, as found for wheat root and shoot Cd in a pot experiment (Yao et al., 2017) and for wheat grain Cd in a field study (Perez and Anderson, 2009). In our study, DGT-available soil Cd was primarily related to soil pH and to a secondary degree to C_{org} and CEC, which is in overall agreement with Perez and Anderson (2009) and He and Singh (1993). Similarly, in a modelling study of wheat grain Cd concentrations in France by Viala et al. (2017), grain Cd could be best predicted by total Cd and pH in the soil solution, CEC and Mn oxides. As indicated by DGT-available Cd and soil organic carbon content in our study, the transfer of soil Cd into wheat shoots decreased in the following order: CONMIN ≥ CONFYM > BIODYN for the DOK and NPK ≥ FYM > COM for the ZOFE trial. In the long-term Broadbalk Wheat Experiment at Rothamsted, grain Cd concentrations were also found to be lower in wheat grown on soil fertilized with farmyard manure than with mineral fertilizers (Jones and Johnston, 1989).

As DGT was the best predictor for Cd phytoavailability but DTPA for Zn we conclude that the two methods should be used complementarily rather than as alternative measures for soil metal availability (Grüter et al., 2017).

In this study, shoot-to-grain transfer factors in all treatments were below one for Cd, while those for Zn were around an order of magnitude higher, indicating that Cd was effectively retained in the shoot tissue, which was also shown in a Cd isotope fractionation experiment by Wiggenhauser et al. (2016). In both trials, grain Cd concentrations were below 60 μg kg^{-1}, which is clearly below the maximum permitted concentration of Cd in wheat grains of 200 μg kg^{-1} according to the UN Codex Alimentarius Commission (FAO/WHO, 1995).

As a result of the differences between treatment effects on the uptake of Zn and Cd, wheat grain Zn/Cd ratios increased with long-term organic fertilizer application. Apart from the negative effect of soil organic matter on Cd availability, plant Zn homeostasis may also be responsible to some extent for the lack of correlation between grain Zn uptake and organic matter addition to soil, as wheat plants can maintain their grain Zn concentrations even at low available soil Zn concentrations (Hacisalihoglu and Kochian, 2003). Apparently, soil Zn supply to wheat was sufficient in all treatments, as indicated by the relatively high DTPA-extractable soil Zn concentrations compared to levels of 0.5 mg kg^{-1} at which soil Zn was reported to be deficient for wheat (Alloway, 2013). However, shoot Zn differed greatly between the two study sites, whereas there was little variation in grain Zn, indicating that in wheat, homeostasis of grain Zn is of higher priority than of Zn in the shoots.

4.3. Zn and Cd balances

When compared to literature values, the calculated Zn (−96 to 1366 g ha^{-1} year^{-1}) and Cd (0.09 to 2.54 g ha^{-1} year^{-1}) balances were in a very similar range (Bengtsson et al., 2003; Keller and Schulin, 2003). As in our work, literature shows that atmospheric deposition and farmyard manure were the most important Zn and Cd inputs.

The Zn and Cd balances presented here have several limitations. Firstly, in the CONFYM treatment of the DOK trial, it is very likely that Zn and Cd applied with organic fertilizers to other crops in the rotation, which was not accounted for in this balance, may also have become available to wheat. Balances for the full crop rotation should be calculated to properly assess the accumulation of heavy metals or mining of essential micronutrients in different farming systems. There was also a high uncertainty in the estimates of atmospheric deposition of Zn and Cd used in the calculation (Keller and Schulin, 2003). The analysis of Zn and Cd concentrations in particulate matter and the measurement of bulk deposition in Switzerland has shown a significant decrease during the last three decades due to emissions mitigation measures (BAFU, 2016).

However, if we want to achieve zero Cd and Zn accumulation in soils, further reductions of Zn and Cd in farmyard manure are necessary, for example through the reduction of the use of Zn for feed additives (Bolan et al., 2004). Not only Zn, but also elevated levels of Cd have been shown to occur in livestock feeds, especially when minerals were used (Nicholson et al., 1999). According to the results of this study, we also strongly recommend limiting the use of high-Cd phosphate fertilizers.

4.4. Conclusions

The investigation of two independent Swiss field trials showed that the long-term application of compost fertilizers reduced the phytoavailability of soil Cd. This was expressed by lower DGT-available soil Cd concentrations and reduced accumulation of Cd in wheat shoots and grains. The concurrent increase in soil organic carbon, soil pH and CEC explain this effect. Due to different uptake behaviour of Zn by wheat compared to Cd, the Zn/Cd ratios of wheat grains, and therefore their quality increased showing long-term compost fertilization can be beneficial for wheat nutritional quality. The calculation of Zn and Cd balances over a wheat cultivation period revealed that large amounts of Zn and Cd enter the agricultural soils via farmyard manure or compost, but also potentially via atmospheric deposition. Moreover, Cd-contaminated P fertilizers also represented a significant Cd input. However, Zn and Cd exports via crop harvest can lead to depletion of both metals in soils if the yields are high.

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Author contributions

The study was conceptualized and designed by all authors; RG and BC conducted all the field work and laboratory analyses; RG did the data analysis, visualization and wrote the original draft of the manuscript; RS and ST reviewed and edited the manuscript; BC, JM, PM, CT and EF contributed corrections and suggestions; CT, EF, RS and ST carried out the supervision. All authors have read and approved this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2019.03.112.

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