The fate of Zn in agricultural soils: A stable isotope approach to anthropogenic impact, soil formation and soil-plant cycling

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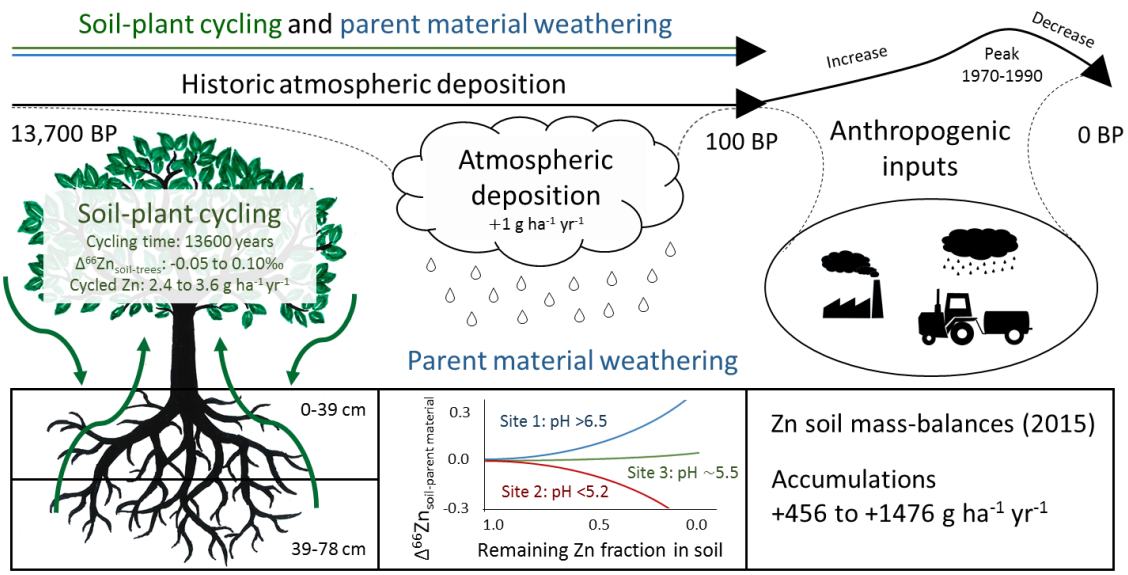
Abstract

The supplementation of Zn to farm animal feed and the excretion via manure leads to an unintended Zn input to agricultural systems, which might compromise the long-term soil fertility. The Zn fluxes at three grassland sites in Switzerland were determined by a detailed analysis of relevant inputs (atmospheric deposition, manure, weathering) and outputs (seepage water, biomass harvest) during one hydrological year. The most important Zn input occurred through animal manure (1,076 to 1,857 g ha^{-1} yr^{-1}) and Zn mass balances revealed net Zn accumulations (456 to 1,478 g ha^{-1} yr^{-1}). We used Zn stable isotopes to assess the importance of anthropogenic impacts and natural long-term processes on the Zn distribution in soils. Soil-plant cycling and parent material weathering were identified as the most important processes, over the entire period of soil formation (13,700 years), while the soil pH strongly affected the direction of Zn isotopic fractionation. Recent anthropogenic inputs of Zn only had a smaller influence compared to the natural processes of the past 13,700 years. However, this will probably change in the future, as Zn stocks in the 0-20 cm layer will increase by 22% to 68% in the next 100 years, if Zn inputs remain on the same level as today.

TOC ART



Introduction

In intensive agriculture, Zn is supplemented to farm animal feed and veterinary drugs to ensure optimal growth and promote wound healing, respectively.[1,2] Feed additives were increasingly supplied to farm animals after intensification of agricultural practices in the 20th century.[3,4] A major part of the Zn is excreted and brought to the field with manure application.[5–9] In the European Union Zn inputs with manure currently range from 300 to 2,700 g ha^{-1} yr^{-1}.[10–12] This Zn might accumulate in soils and compromise the growth of microorganisms, invertebrates, and plants.[13,14]

In uncontaminated environments, Zn is naturally introduced to soils by the weathering of parent materials. The bioavailable and exchangeable Zn^{2+} is mainly adsorbed to soil organic matter and (hydr)oxides, and desorption processes into soil solution depend on the pH value.[15] Nonspecific sorption to clay minerals and mineral precipitation are important at elevated Zn concentrations and are only weakly related with the pH value.[15] Typical soil Zn concentrations range from 10 to 100 mg kg^{-1} [15] and correspond to Zn stocks of ~45,000 to 600,000 g ha^{-1} in the uppermost 50-cm soil layer. Another relevant Zn source to soils can be atmospheric deposition. Zn emissions to the atmosphere originate from natural sources like biological or geogenic particles, volcanoes or forest fires[16] and from human sources like fossil

fuel combustion, metal production, and refuse incineration.[17] In Europe, anthropogenic Zn emissions increased during the 20th century, peaked in 1965-1970 with ~30x higher deposition rates than natural background deposition rates, and decreased thereafter.[18,19] Recent studies have reported atmospheric deposition rates between 20 and 540 g ha^{-1} yr^{-1} in 13 European countries.[20]

The dominant Zn outputs from agricultural soils are leaching with seepage water and crop harvest. Zinc concentrations in soil solution can be estimated with the help of soil-water partition coefficients (K_d values) which predict an increase of dissolved Zn concentrations by a factor of 5 per unit pH decrease.[21] For European soils, leaching rates between 18 and 135 g ha^{-1} yr^{-1} have been reported.[11,12,22,23] The Zn outputs with harvest mainly depend on crop type, soil productivity and soil Zn concentrations with reported values for European soils between 30 and 540 g ha^{-1} yr^{-1}.[10–12,23]

Natural abundance stable isotope compositions are a tool that is increasingly used to differentiate between geogenic and anthropogenic sources.[24] Igneous and sedimentary rocks show limited variations in their isotopic compositions (δ^{66}Zn = -0.22 to 0.28 ‰), except carbonates (0 to 1.13 ‰)[25] which can be more enriched in heavy isotopes, caused by the preferential recycling of isotopically light Zn in the water column by microorganisms. In contrast, anthropogenic activities cause larger isotopic variability (-0.98 to 0.53 ‰) mainly produced by evaporation and condensation processes of elemental Zn in metal refineries.[26,27] Because of the distinct isotope composition of geogenic and anthropogenic sources, previous studies successfully traced Zn contamination sources of soils, such as Zn from smelters,[28] but also from sources with less isotopic variability like diffuse anthropogenic contamination[29] and pig slurry.[30] Beside different sources, biogeochemical processes can also produce significant Zn isotope fractionation in soils and plants.[31,32] The difference in isotope composition between bulk soil and the plant shoot is a result of isotope fractionation processes during Zn desorption from the bulk soil into soil solution, Zn speciation in soil solution, Zn plant uptake and Zn transport from root to shoot. During weathering of biotite granite[33] and sulfide-rich minerals,[23] leachates showed partly heavier and partly lighter Zn isotopic composition, compared to the parent rocks. Another study on the formation of aerobic soils on the island of Maui attributed the slight depletion of the soils in heavy Zn isotopes to permanent leaching of organic acid-bound Zn.[34] Furthermore, several studies found the preferential inner-sphere

adsorption of heavy Zn isotopes onto (hydr)oxides ($\Delta^{66}Zn_{aqueous\text{-}adsorbed}$ = -0.29 to -0.52 ‰),[35,36] quartz and amorphous SiO_2 (-0.60 to -0.94‰),[37] calcite (-0.41 to -0.73‰),[38] kaolinite (-0.49 ± 0.06‰),[39] and high-affinity organic matter sites (-0.24 ± 0.06‰).[40] In contrast, Zn adsorption as outer-sphere complex and to low affinity organic matter sites exhibited much smaller or no isotopic fractionation.[37,39,40] Soil leachates showed distinct isotopic fractionation. While 0.1 M HCl extracts were mostly isotopically heavier than the respective agricultural bulk soils, 0.01 M $CaCl_2$ extracts were isotopically lighter than plant-free Zn-contaminated bulk soils ($\Delta^{66}Zn_{extract\text{-}soil}$ = -0.07 to -0.25‰),[41,42] but became isotopically heavier in the presence of plants. The latter was explained by plant-induced soil acidification and mobilization of the (hydr)oxide-bound Zn pool.[41,43,44] In soils with sufficient Zn supply, plant shoots were more enriched in light isotopes than plant-available soil pools,[41,42,45,46] but partly more enriched in heavy isotopes than bulk soils.[46,47] This variation can be explained by plant-induced Zn mobilization (isotopically heavy), low-affinity Zn uptake (isotopically neutral to light), complexation, and storage of excessive cytosol Zn in vacuoles of root cells (isotopically heavy), and the Zn transport to the shoot through efflux transporters (isotopically light).[32,48]

Several studies established Zn mass balances for agricultural soils and identified manure as the most important input which leads to net Zn accumulations in European soils.[11,20,49–51] Nevertheless, these studies did not include fluxes with atmospheric deposition and seepage water or estimated them with model predictions. Fekiacova et al.[30] hypothesized that isotopically heavier topsoils were related to past pig slurry inputs. However, the study partly used literature data and did not measure isotope compositions of all relevant system fluxes.[30]

The addition of Zn from the atmosphere or via manure results in a preferential accumulation of Zn in the topsoil, because of the low Zn mobility in most soils. Therefore, Zn accumulation in the A horizon relative to the subsoil and parent material is usually interpreted as indication of anthropogenic metal accumulation.[52,53] However, in recent work we have shown that the accumulation of Cd in topsoils of three Swiss arable soils mainly originated from natural soil-plant cycling during the whole Holocene[54] as also described for growth-limiting plant nutrients.[55,56]

Here, we used *in situ* measured data to establish Zn mass balances for three grassland sites. Soil Zn concentrations and relevant Zn inputs (atmospheric deposition, manure, weathering of parent material) and outputs (seepage water, grassland harvest) were determined during

111 one hydrological year, from May 2014 to May 2015. Additionally, natural variations in Zn
112 stable isotope compositions were used to trace sources and to investigate processes in the
113 soils.

114 The aims of this work were to: (i) determine Zn accumulation rates in grassland soils under
115 current agricultural practices, (ii) differentiate between geogenic and anthropogenic Zn in the
116 soils, and (iii) identify important Zn redistribution mechanisms in the soils.

Materials and Methods

Study Sites

The study was carried out at three grassland monitoring sites (Figure S1) of the Swiss Soil Monitoring Network (NABO)[9] at Tänikon (TA), Ebikon (EB), and Ependes (EP). The sites have been used as grasslands for at least the last 30 years and were chosen because of contrasting geology and soil properties. The soils developed on alluvial deposits (TA), sandstone (EB), and moraine material (EP). All three soils were classified as Eutric Cambisols. During the time of the field sampling, the sites were managed in the same way like in the years before our experiment. The grassland sites at TA and EB were cut 4 and 6 times during our study period, respectively, and the grassland site at EP was used for grazing of cows (Table S3). Liquid cow manure was applied at all sites, liquid pig manure was applied at TA and EB, and poultry dung was applied at EP every fourth year (Table S3).

Sampling

Soil samples were taken from five depths (0-10 cm, 10-20 cm, 20-50 cm, 50-75 cm, and >75 cm, Figure S2). Inputs and outputs (Figure 1) were sampled during one hydrological year from May 2014 to May 2015. Soil parent material was collected at each site. The C horizons at TA (60-80 cm) and EP (120-140 cm) were obtained from the NABO[9] whereas sandstone at EB and moraine material at EP were sampled from nearby outcrops. Cumulative volumes of atmospheric deposition and seepage water were stored in canisters in the field and sampled every second week. The volumetric water content of the soils was determined with 1-h resolution by time domain reflectometry at 50 cm depth. Homogeneous liquid manure samples were taken four times a year at each site. During the growing season (2014.w28 to 2014.w44 and 2105.w14 to 2015.w24), aboveground biomass was randomly sampled in triplicates at each site every fourth week.

Laboratory Analysis

Basic soil properties including pH, cation-exchange capacity (CEC), texture, bulk density, coarse soil content (> 2mm), and C, N, and S concentrations were analyzed and the soils were characterized according to the World Reference Base for Soil Resources (Table S1).[57]

Aliquots of seepage water samples were used to measure major cations (Na^+, NH_4^+, K^+, Mg^{2+} and Ca^{2+}) and anions (Cl^-, NO_2^-, NO_3^-, PO_4^{2-} and SO_4^{2-}) by ion chromatography (IC, DX-120, ThermoFisher Scientific, Waltham, MA, USA). Accordingly, dissolved organic carbon (DOC) was measured with a vario TOC cube analyzer (Elementar Analysensysteme, Langenselbold, Germany). Total dissolved concentrations of the most abundant elements (Al, V, Mn, Fe, Co, Ni, Cu, Zn, As, Rb, Sr, Ba, and U) in atmospheric deposition and seepage water were measured by inductively-coupled mass spectrometry (ICP-MS, 7700x, Agilent Technology, Waldbronn, Germany) and used later for Zn speciation modeling.

The stable Zn isotope compositions of all samples were determined with standard-sample bracketing and Cu doping for instrumental mass-bias correction by multiple collector inductively-coupled plasma mass spectrometry (Neptune Plus™ High Resolution MC-ICP-MS, Thermo Fisher Scientific, Waltham, MA, USA) at the Geological Institute of the University of Bern (SI 1.3). The total procedural Zn blank for the isotope analysis of parent materials, soils, aboveground biomass, and manure samples (0.7 to 6.3 ng) accounted for less than 0.2% of the native Zn mass in the samples. The total procedural blanks of seepage water (71 to 75 ng) and bulk deposition (218 to 223 ng) accounted on average for 1.3% and 4.5% of the total Zn masses (SI 1.3). Several standard reference materials (SRMs) were analyzed for quality control (Table S2). The Zn isotope composition of ryegrass (BCR-CRM281) was slightly heavier than formerly reported values.[47,58,59] All other standards (USGS BCR-2 and CMI SCL7003) showed robust results in agreement with published values.[60]

The Zn stable isotope compositions of the samples were reported relative to the IRMM 3702 Zn isotope reference material using a δ notation based on the $^{66}Zn/^{64}Zn$ ratio (Equation S1). The analytical error was 0.07‰, i.e. two times the standard deviation of the measured IRMM-3702 standards (n=11). Two samples were considered significantly different in their isotopic composition if their error bar (2 standard deviations of the repeated analysis of each sample) did not overlap. The $\Delta^{66/64}Zn$ value, which denote the apparent isotopic fractionation between two reservoirs and/or two fluxes (e.g. between soil and seepage water) were calculated according to Equation S3.

174

175 *Modeling and Calculations*

176 Zn speciation in seepage water was calculated with Visual MINTEQ. The pH, cation, anion,
177 total element, and dissolved organic matter (DOM) concentrations in soil solution were used
178 as input data. Ionic strength was calculated by Visual MINTEQ. Dissolved organic matter
179 concentrations were inferred from the measured dissolved organic C concentrations (DOC),
180 using a conversion factor DOM:DOC of 2:1.[61] Zinc speciation was modelled with two
181 scenarios, because it has been reported that the chemical DOM composition was an
182 important source of uncertainty.[62] DOM in the min-HS (HS = humic substances) scenario
183 consisted of 2% humic acids (HA), 18% fulvic acids (FA), and 80% hydrophilic organic
184 substances and in the max-HS scenario of 6% humic acids, 54% fulvic acids and 40%
185 hydrophilic organic substances, in line with reported values.[62,63]

186 Zinc and Ti concentrations in the parent materials and soils were used to calculate Zn mass
187 gains or losses per unit volume of soils relative to parent materials (τ_{Zn} values and remaining
188 Zn fractions $f_{Zn} = \tau_{Zn}+1$, SI 1.4).[64] Zn mass balances (SI 1.5) were calculated for each study soil
189 in the upper soil layer (0-50 cm, Figure 1, Table S3), considering inputs from weathering,
190 atmospheric deposition, and manure as well as outputs with seepage water, and
191 aboveground biomass. Input from weathering was estimated as dissolution of the coarse soil
192 (> 2mm) which introduced Zn to the fine earth.

193 We used three models to investigate the Zn (isotope) distribution in the soils, an
194 *anthropogenic and atmospheric impact*, a *soil-plant cycling*, and a *Rayleigh fractionation*
195 model for soil formation. The outputs of the models were described in Cases A, B, and C for
196 which f_{Zn} and δ^{66}Zn values of the soils were calculated. Overall, a combination of the models
197 was used to explain the depth distribution of the Zn concentrations and stable isotope ratios
198 in the soils. Case A was the starting point of the model calculations, using current values in the
199 year 2015 including anthropogenic impacts, atmospheric deposition, and soil-plant cycling.
200 From there, the *anthropogenic and atmospheric impact model* was used to assess the
201 importance of anthropogenic and atmospheric fluxes for the Zn stocks and Zn isotope
202 compositions of the soils (SI 1.6). These calculations were based on measured Zn fluxes and
203 isotope compositions as well as on literature data on past manure inputs and atmospheric

deposition.[10,12] The output of this model was **Case B**, desribing the state in the year 1915 without anthropogenic impact and atmospheric deposition. To arrive at this case, estimated past outputs were added and estimated past inputs subtracted from the current bulk soil Zn stocks (Equation S8) and bulk soil isotope compositions (Equation S9). Next, the *soil-plant cycling model*[54] was used to investigate if trees, the potential native vegetation which formerly covered the agricultural soils, took up Zn from the deeper soil horizons and added it to the upper soil horizons, over the time of soil formation. Thereby, the soils were first subdivided into two layers (0-39 cm and 39-78 cm). For these two layers, we derived the remaining Zn fractions ($f_{Zn} = \tau_{Zn} + 1$) and the $\delta^{66}Zn$ values by averaging the respective values of five bulk soil layers 0-10 cm, 10-20 cm, 20-50 cm, 50-75 cm, >75 cm from **Case B**. Furthermore, the potential Zn surplus in the upper (0-39 cm) relative to the deeper (39-78 cm) soil layer, presumably cycled by trees,[54] was calculated with the help of τ_{Zn} values. This potential Zn surplus was divided by the age of the soils (13,600 yearsin 1915)[65–67] to obtain the annually cycled Zn. Next, the annually cycled Zn mass was subtracted from the upper and added to the deeper soil-layer, in 13,600 steps to incrementally reverse the assumed process during the soil formation before the start of intensified agriculture. Additionally, the change in Zn isotope composition of the two soil layers was calculated (Equations S10 and S11) by subtracting the Zn isotope composition of the annually cycled Zn from the upper and adding it to the deeper soil-layer in the same 13,600 annual steps. The isotope composition of the cycled Zn was determined for each calculation step using the measured $\Delta^{66}Zn_{\text{soil-aboveground biomass}}$ values at the three study sites (SI 1.7). After 13,600 calculation steps, the f_{Zn} and $\delta^{66}Zn$ values were averaged for the two soil-layers and represented **Case C**, i.e. the state in the year 1915 without anthropogenic and atmospheric impact and without soil-plant cycling. Error propagation was calculated according to Imseng et al.[54] Finally, the f_{Zn} and $\delta^{66}Zn$ values of **Case C** were used in a *Rayleigh fractionation model* to describe the soil isotope composition change during parent material weathering and Zn leaching with seepage water (Equation 1).

$$Equation\ 1: \quad \Delta^{66}Zn_{soil\text{-}parent\ material} = \varepsilon \ln f_{Zn}$$

where $\Delta^{66}Zn_{\text{soil-parent material}}$ is the difference in the $\delta^{66}Zn$ value between a soil layer and its parent material and ε is the Rayleigh fractionation factor for soil formation.

Results

Our mass balances revealed that the current Zn inputs were 2 to 4 times higher than Zn outputs. Consequently, the Zn mass balances showed net accumulations at all three sites, which were the highest at TA (1,476 g ha^{-1} yr^{-1}) followed by EB (1,070 g ha^{-1} yr^{-1}) and EP (456 g ha^{-1} yr^{-1}, Figure 1, Table S3). The quantitatively most important Zn input was manure application (1,078 to 1,857 g ha^{-1} yr^{-1}) which accounted for more than 98% of the total Zn inputs at all three sites. In contrast, Zn inputs from atmospheric deposition (20 to 39 g ha^{-1} yr^{-1}) and weathering (0.01 to 8.6 g ha^{-1} yr^{-1}) were considerably smaller. Outputs with aboveground biomass (315, 312 and 585 g ha^{-1} yr^{-1}) exceeded outputs with seepage water (99, 16 and 55 g ha^{-1} yr^{-1}) at TA, EB and EP, respectively.

Parent material isotope compositions ranged from -0.07‰ to 0.07‰ at the three sites (Figure S3, Table S3). The soil at TA was on average isotopically slightly heavier ($\Delta^{66}Zn_{soil(0\text{-}75cm)\text{-}C\ horizon}$ = 0.07 ±0.07‰) and the soil at EB ($\Delta^{66}Zn_{soil(0\text{-}75cm)\text{-}sandstone}$ = -0.11 ±0.07‰) isotopically lighter than the parent materials. In contrast, the soil at EP ($\Delta^{66}Zn_{soil(0\text{-}75cm)\text{-}parent\ materials}$ = 0.00 ±0.07‰) was on average isotopically not different from the parent materials. The Zn isotopic compositions of the inputs and outputs were on average little different from the soils or isotopically lighter (Figure S3, Table S3). First, the manure was more enriched in light Zn isotopes than the soil at TA ($\Delta^{66}Zn_{soil(0\text{-}75cm)\text{-}manure}$ = 0.14 ±0.07‰), but slightly more enriched in heavy isotopes than the soils at EB (-0.06 ±0.07‰) and EP (-0.05 ±0.07‰). Second, the atmospheric deposition was on average more enriched in light Zn isotopes than the soils at TA ($\Delta^{66}Zn_{soil(0\text{-}75cm)\text{-}atm.\ dep.}$ = 0.32 ±0.03‰), EB (0.23 ±0.07‰), and EP (0.13 ±0.07‰). Third, compared with the soils, seepage water was on average Zn-isotopically lighter at TA ($\Delta^{66}Zn_{soil(0\text{-}75cm)\text{-}seepage\ water}$ = 0.59 ±0.05‰) but Zn-isotopically heavier at EB (-0.11 ±0.07‰) and EP (-0.10 ±0.07‰). Finally, the aboveground biomass was slightly more enriched in light Zn isotopes than the soil at TA ($\Delta^{66}Zn_{soil(0\text{-}75cm)\text{-}aboveground\ biomass}$ = 0.09 ±0.07‰) and Zn-isotopically identical to the soil at EB (0.00 ±0.07‰) and EP (-0.06 ±0.07‰).

Discussion

Zn Mass Balances

Input Fluxes. The size of the Zn input with manure was related to the type of manure, because Zn concentrations in pig manure (384 to 962 mg Zn kg dry weight^{-1}) were higher than Zn concentrations in cow manure (133 to 311 mg Zn kg dry weight^{-1}). The proportion of pig manure of the total addition of manure (dry weight, Table S3) was bigger at TA (19%) than at EB (6%) whereas only cow manure and poultry dung were spread at EP. Therefore, Zn input with manure was highest at TA, followed by EB and EP (Figure 1). The Zn inputs with manure in our study fell into the range of reported values for European agricultural soils (300 to 2,700 g ha^{-1} yr^{-1}).[10–12] Atmospheric deposition of Zn was by one to two orders of magnitude smaller than Zn inputs with manure and varied only by a factor of two among the sites (19.5 to 38.8 g ha^{-1} yr^{-1}). The deposition rates were below reported values for Swiss agricultural soils in 2003 (80 to 130 g ha^{-1} yr^{-1})[10] and in the lower range of atmospheric deposition rates for European soils (20 to 540 g ha^{-1} yr^{-1}).[20] The low deposition rates of Zn in our study might be related to the rural location of our sites and the recently decreasing anthropogenic Zn emissions to the atmosphere.[17,18,68] Zinc input from weathering was the highest at TA (0.5% of the total Zn inputs) and negligible at EB and EP. The comparatively high contribution of Zn from weathering at TA can be attributed to the calcareous coarse soil for which reported weathering rates[69] are three orders of magnitude higher than for the silicatic coarse soils[70,71] at the two other sites.

Output Fluxes. Despite similar amounts of harvested biomass among the sites, Zn output with aboveground biomass was about 1.6 times higher at EP than at TA and EB, mainly because of the higher average Zn concentrations in aboveground biomass at EP (Table S3). The Zn concentrations in aboveground biomass and Zn outputs with aboveground biomass were in the range of recently reported values.[10,22,72] The decreasing Zn outputs with seepage water from TA via EB to EP can be explained by the declining Zn concentrations (Table S2). Our results are at the lower end of reported leaching rates for European soils (18 and 135 g ha^{-1} yr^{-1}).[11,12,22,23]

Balance. The most influential driver for the Zn mass balance of the soils was manure application, [73] causing a considerable net accumulation of Zn in all three soils. The predicted accumulation agrees well with recently reported trends for Zn accumulation on intensively used grassland soils.[9]

294 *Origin of the Zn in the Soils*

295 The Zn distribution in the grassland soils is a result of different Zn inputs (manure,
296 weathering, and atmospheric deposition), Zn outputs (aboveground biomass, seepage water),
297 and soil formation processes. To better understand the origin of the Zn in the soils, the Zn
298 isotope fluxes are discussed in a first step. Subsequently, the stable Zn isotopes were used in
299 our *anthropogenic and atmospheric impact model*, to assess the importance of anthropogenic
300 inputs for the Zn distribution in the soils.

301 *Zn isotope fluxes.* The isotopic composition of parent materials (Figure S3, Table S3) was in the
302 range of formerly measured values for crustal rocks and minerals (-0.22 to 0.28‰).[74,75]
303 Furthermore, the soils showed only little Zn-isotopic differences (at most 0.13‰ lighter or
304 0.10‰ heavier) from the parent materials. This indicates, that weathering only marginally
305 influenced Zn-isotopic compositions of the soils, even though between 12% and 58% (except
306 0-10 cm at EB) of the initial Zn was lost from the soils (revealed by τ_{Zn} values), probably mainly
307 with seepage water. Similarly, a study on Zn isotope fractionation during soil development
308 found on average small differences in stable Zn isotope ratios between parent material and
309 soil ($\Delta^{66}Zn_{\text{parent material-soil}} \leq 0.20$‰) at Zn losses between 0 and 95% suggesting a generally weak
310 influence of soil formation on Zn isotopic compositions.[34]

311 The average isotopic composition of manure was similar to the isotopic composition of the
312 aboveground biomass. One reason for this could be that Zn in feed additives was isotopically
313 little different from Zn in the aboveground biomass and that digestion in the farmland animal
314 digestive tract caused only minor isotopic fractionation. Alternatively, digestive processes
315 might have caused isotopic fractionations but were counterbalanced by an isotopic signature
316 of the feed additives which deviated from that in the basic feed in the opposite direction than
317 this fractionation. Isotopic compositions of recently analyzed pig slurries (-0.11 and -
318 0.08‰)[30] were slightly lighter than or identical to our values.

319 The similar isotopic compositions of the atmospheric deposition among the three sites
320 indicate similar Zn sources or sources with similar $\delta^{66}Zn$ values. This Zn might partly derive
321 from natural sources because its isotope composition is in the range of rural rain water
322 samples (-0.32 to -0.17‰),[76] igneous rocks and minerals (-0.22 to 0.28‰),[30,74,75] aboveground
323 plant material (-0.50 to 0.50‰),[25] and aerosols from the North Atlantic (-0.29 to -0.15‰).[77]

Alternatively, the Zn could also originate from anthropogenic sources, because bus air filters in urban areas (-0.22 to -0.28‰) and waste incineration flue gas samples (-0.25 to -0.13‰) showed similar isotopic compositions.[78] In contrast, smelting processes and gasoline combustion seemed to be of less importance at our sites as dust from smelter chimneys (-0.99 to -0.95‰)[26,27] and aerosols from urban areas (-1.41 to -0.41‰)[79] were isotopically lighter.

The isotope fractionation between soil and seepage water was different among the three sites. At TA, the site with the highest pH and ionic strength, Zn in seepage water was much lighter than at EB and EP, the sites with lower pH and ionic strength. Several studies investigated the isotopic fractionation during Zn adsorption to quartz and amorphous SiO_2,[37] kaolinite,[39] (hydr)oxides,[35,36,80] organic matter,[40] and calcite[38] and found stronger enrichments of heavier isotopes with increasing specificity of the bonds (inner-sphere vs. outer-sphere; high-affinity vs. low affinity; tetrahedral vs. octahedral). This can be explained by the bond lengths of Zn with its neighboring atoms, which become shorter with increasing specificity.[35,37] Thereby, increasing pH and ionic strength drove the Zn to bind to more specific adsorption sites and hence led to stronger enrichments of heavy isotopes at the surfaces while leaving the solutions isotopically lighter.[35–38,40] In our study, the higher pH at TA than at EP and EB might have favored a more pronounced specific binding of Zn and caused the depletion of heavy Zn in the seepage water at TA. Furthermore, the higher Mg^{2+} and Ca^{2+} concentrations in soil solutions at TA (18 ±4 mg L^{-1} and 77 ±19 mg L^{-1}) than at EB (2.3 ±0.4 mg L^{-1} and 17 ±3 mg L^{-1}) and EP (1.5 ±0.4 mg L^{-1} and 24 ±6 mg L^{-1}) might have been important competitors of Zn for the less specific binding sites at the mineral surfaces in soil. As a consequence, the preferential binding of isotopically heavy Zn to the more specific binding sites might have been further enhanced. This hypothesis is supported by the Zn speciation modeling, which showed that between 61% (max-HS scenario) and 90% (min-HS scenario, Table S4) of the dissolved Zn was present as free Zn^{2+} and only a minor part complexed by organic acids while other Zn species only occurred in negligible concentrations. Hence, the potential enrichment of isotopically heavy Zn in Zn-DOM complexes seems to be only of minor importance at our sites.

The stronger enrichment of light isotopes in the aboveground biomass than in the soil at TA might be a consequence of the isotopically light Zn in soil solution, from where plants take up

their nutrients. In contrast, at EB and EP, the sites with a Zn-isotopically slightly heavier soil solution than bulk soil, no isotopic difference was measured between aboveground biomass and bulk soil. Several studies investigated Zn uptake and translocation mechanisms with the help of Zn stable isotopes. Thereby, low affinity uptake, which is expected to dominate at Zn concentrations above 10 nmol L^{-1} (like at our sites),[81] was associated with no isotopic fractionation or the preferential uptake of light isotopes.[32,44,82,83] Furthermore, Zn translocation in plants was linked with the preferential retention of heavy isotopes in roots and transport of light isotopes to aboveground plant tissues.[32,84] At our sites, aboveground biomass was isotopically not distinct or heavier than seepage water. This might be a consequence of the purposeful acidification of the rhizosphere soil by the plants to mobilize nutrients, particularly phosphorus. As side effect, also micronutrients like Zn were mobilized and rendered the plant-available Zn isotopically heavier, like it was reported for *Agrostis capillaris* L. on Zn-contaminated soils.[41] This effect was strongest at TA, the site with the highest pH and highest $\Delta^{66}Zn_{soil\text{-}seepage\ water}$ value.

Anthropogenic and atmospheric impact on the Zn in soils. To be able to investigate natural Zn redistribution processes in soils, former anthropogenic and atmospheric impacts on the soil Zn concentrations were mathematically removed with the *anthropogenic and atmospheric impact model*. For that model, we simplified our agricultural systems and assumed that they were only anthropogenically influenced during the last 100 years, which is justified by two reasons. First, Zn concentrations in Alpine snow and ice core samples correlated with anthropogenic Zn emissions and indicated that atmospheric deposition rates were 30 times lower during the 18th and 19th centuries than in the 1970s.[18,19] Further, even in the 18th and 19th century, only 14% of the deposited Zn was of natural origin. Thus, atmospheric deposition rates before the industrialization can be expected to be 200fold lower than in the 1970s, i.e. ~1 g ha^{-1} yr^{-1}. Second, feed additives and Zn input with manure became more important after World War I, with intensification of agricultural practices and the use of concentrated animal food.[3,4] Before that time, annual Zn fluxes with fertilization and harvest were considerably smaller than during the 20th century. Therefore, in our study, soils were considered as closed systems regarding the Zn fluxes.[3]

For the further investigations of natural soil formation processes, the soils were subdivided into two layers (0-39 cm and 39-78 cm) and τ_{Zn} as well as Zn isotope compositions were

386 averaged for both layers. In that way, also the influence of anthropogenic activities and
387 former atmospheric deposition could be estimated for these two soil layers. For **Case A**
388 (referring to the year 2015), the remaining Zn fractions (f_{Zn}) were higher in the upper (0-39
389 cm) than in the deeper (39-78 cm) soil layers (Figures 2, 3a, S4, S5). This Zn distribution was
390 partly caused by anthropogenic inputs and historic atmospheric inputs. According to our
391 model calculations, anthropogenic impacts (16%, 18% and 6%) and historic atmospheric
392 deposition (4%, 4% and 4%) increased the Zn stocks in the upper soil layer (0-39 cm) at TA, EB
393 and EP, respectively. For the deeper soil layer (39-78 cm), these increases were considerably
394 smaller at all three sites accounting for only 2% of the stocks. However, even without
395 anthropogenic impact and former atmospheric deposition (**Case B** referring to the year 1915),
396 f_{Zn} values are higher in the upper (0-39 cm) than in the deeper soil layer (39-78 cm), which
397 will be discussed in the next chapter. As outlined above, the Zn stocks and Zn isotopic
398 compositions were only marginally influenced by humans before 1915. Furthermore, the
399 historic atmospheric inputs have been considered and subtracted. For these reasons, **Case B**
400 can be used to further investigate Zn redistribution processes in soils.

401

402 *Zn Redistribution Processes in the Soils*

403 As mentioned above, even without anthropogenic impact and former atmospheric inputs
404 (**Case B**), remaining Zn fractions (f_{Zn}) were higher in the upper (0-39 cm) than in the deeper
405 (39-78 cm) soil layers (Figures 2, S4, S5, 3b, 3c). However, if leaching with seepage water was
406 the only important process during soil formation we would expect the upper older soil
407 horizons to be more depleted in Zn than the younger deeper soil horizons. Several studies
408 revealed the importance of the plant pump for the distribution of nutrients[55,56] and metals in
409 soils.[54,85–87] Here, we applied a soil-plant cycling model[54] to assess the importance of the plant
410 pump for the Zn distribution in the studied soils. After removal of the soil-plant cycling effect
411 (**Case C**), the remaining Zn fractions were smaller in the upper than in the deeper soil-layer
412 (Figures 2, S4, S5, 3d). Furthermore, by subtracting the influence of soil-plant cycling, it was
413 possible to fit Rayleigh-type fractionation models between soil solid phase and soil solution
414 for the three soils. These models resulted in different apparent fractionation factors during
415 weathering and soil formation (ε) of -0.18, 0.18 and 0.00 at TA, EB and EP, respectively (Figure

3d), suggesting that different pedogenetic Zn-fractionation processes were at play at the three study sites. The finding that the Zn isotope fractionation derived from the Rayleigh-type model differed among the study sites deviated from our previously reported similarly derived finding for Cd.[54] In contrast to Zn, the Cd isotope fractionation between solid soil and seepage water was constant among the sites, despite differing soil pH values.[88] For Zn, the different directions of the isotope fractionation coincided with different soil pH values at the three sites (Figure 3e). While these patterns can be interpreted independently for the three different soils, which formed from different parent materials, it can also be assumed that the three soils follow one overall pattern controlling the Zn isotope fractionation in soils depending on the pH. Hypothesizing one overall pattern, the sequence might start at TA and end at EB. With the presence of carbonates and an alkaline soil pH like at TA, isotopically light Zn was leached with seepage water and shifted the isotope composition of the soil towards heavier values. With the depletion of the carbonate buffer and a slightly acidic soil pH like at EP, isotopically heavy Zn was leached from the soil and shifted its isotope composition toward lighter values thereby compensation the previous fractionation in the other direction, which might explain the isotopic similarity of Zn in bulk soil and parent material. With ongoing soil acidification like at EB, heavy Zn isotopes were continuously leached and caused the bulk soil to be isotopically lighter than the parent material. However, our hypothesis of this overall pattern would need further confirmation from additional soils.

Environmental Implications

The most influential driver for the Zn mass balance of the soils was manure application.[73] The current Zn concentrations in the topsoils (85 to 122 mg kg^{-1}, Table S1) might already be toxic for some plants,[89] invertebrates,[90] and microorganisms.[91] If current agricultural practices continue in the future, soil Zn concentrations will further increase. Assuming the same Zn addition of inputs and Zn removal of outputs from the different soil layers like in the anthropogenic and atmospheric impact model (SI 1.6), the largest part of Zn would remain in the uppermost soil layer (0-20 cm) and the Zn stock of this horizon would increase by 68%, 36% and 22% within 100 years at TA, EB and EP, respectively. This represents a faster accumulation than in the last 100 years. Between 1915 and 2015, the Zn stocks in the

uppermost soil layer (0-20 cm) increased only by 30%, 26% and 8% at TA, EB and EP, respectively, because of the lower Zn inputs with manure. Over the millennia of soil development, soil-plant cycling, mineral weathering, and leaching were more relevant than the anthropogenic Zn inputs of the past few decades. However, under the current agricultural practice the anthropogenic impact will probably overprint the natural Zn distribution in soils, causing possible harmful Zn concentrations in the surface soils. The predicted accumulation supports recently reported trends for Zn accumulation on intensively used grassland soils.[9] To prevent further Zn accumulation in soils, it is crucial that Zn in feed additives for farm animals are not above the required doses and that the quantity of manure applied to a specific area is limited. According the European Food Safety Authority (EFSA), the authorized maximum Zn concentrations in animal feed could be reduced by 20% without compromising health, welfare, and productivity of the target animals.[72] Furthermore, the use of phytase could enhance the Zn availability for non-ruminants and allow for a reduction of even 30% of the currently authorized maximum concentrations for pigs.[72]

Supporting information

Section 1: Detailed information on materials and methods. Section 2: Location of the study sites. Section 3: Sampling design. Section 4: Isotope compositions of all measured samples. Section 5: Soil-plant cycling models for TA and EP. Section 6: Soil properties. Section 7: Standard reference materials. Section 8: Zn mass balance and anthropogenic impact model. Section 9: Seepage water properties.

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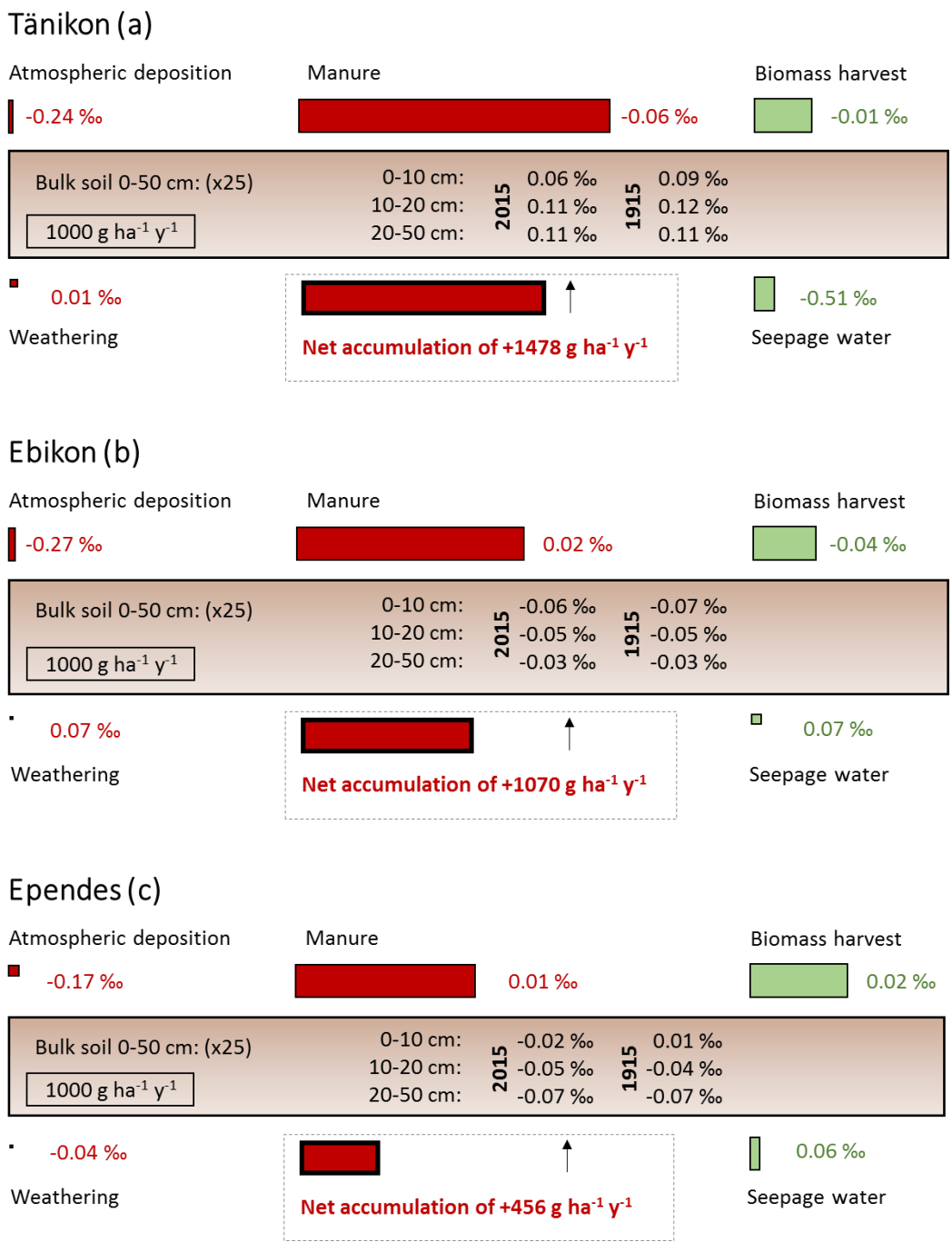
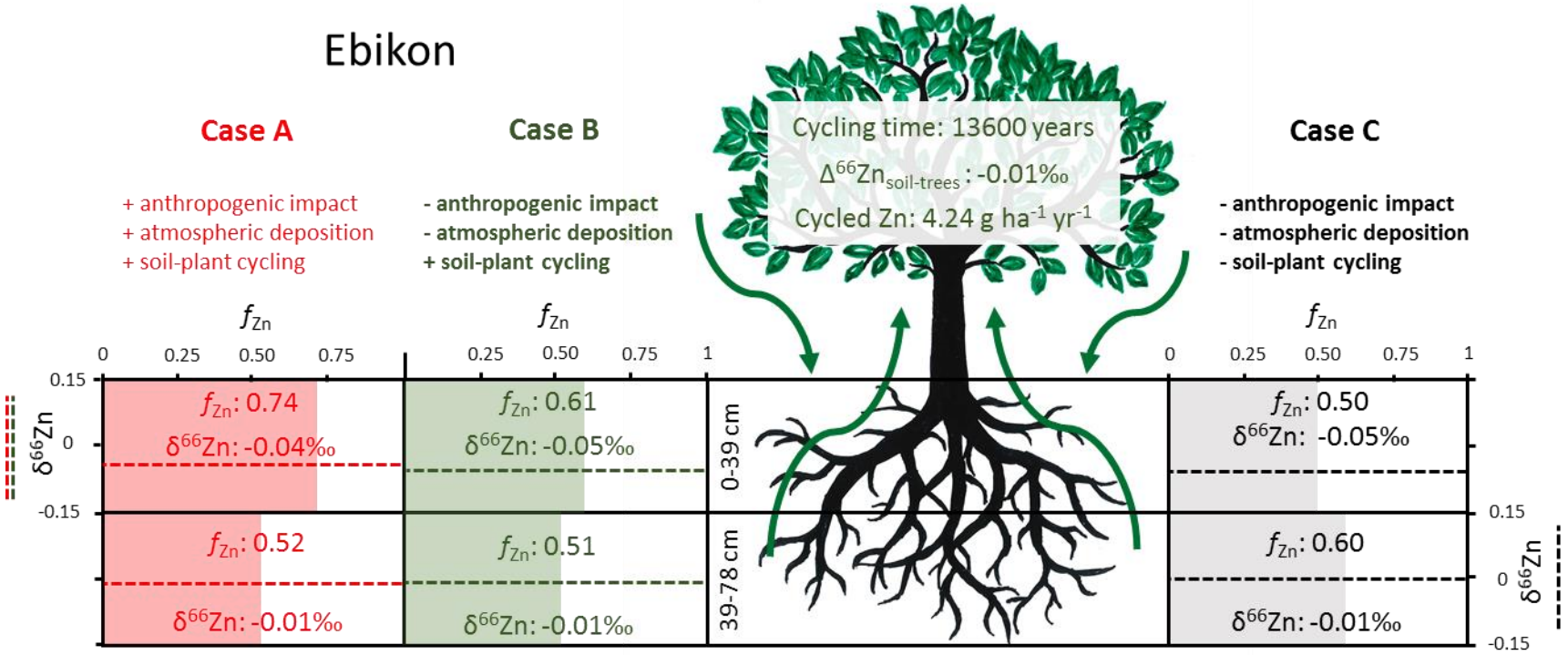


Figure 1. Zinc mass balance of the three grassland sites Tänikon (a), Ebikon (b), and Ependes (c) in Switzerland for one hydrological year (May 2014 – May 2015). System inputs are shown in red; system losses are shown in green. Sizes of the boxes are proportional to the size of Zn fluxes (compared to the reference box of 1000 g Zn ha^{-1} yr^{-1}). Sizes of the bulk soil boxes had to be reduced and would be 25x bigger to proportionally represent real Zn stocks of the soils. Net accumulations represent the mass balance values after one hydrological year. The $\delta^{66}Zn$ values of inputs and outputs are given next to the boxes. The $\delta^{66}Zn$ values of the three soil layers are given in the soil boxes; 2015 values were measured, 1915 values were calculated with the "anthropogenic impact model".



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774 Figure 2. Results of the *anthropogenic and atmospheric impact* and the *soil-plant cycling model* at Ebikon. **Case A** in red gives the remaining Zn fractions (f_{Zn} =
775 τ_{Zn} + 1) and the isotope compositions ($\delta^{66}Zn_{soil}$) of the two soil-layers (0–39 and 39–78 cm) in the year 2015 and includes the anthropogenic impact, former
776 atmospheric deposition, and soil-plant cycling. **Case B** in green gives the f_{Zn} and $\delta^{66}Zn_{soil}$ values after application of the *anthropogenic and atmospheric impact*,
777 and *soil-plant cycling model* in the year 1915, without anthropogenic impact, without former atmospheric deposition but with soil-plant cycling. **Case C** in black
778 gives f_{Zn} and $\delta^{66}Zn_{soil}$ values after application of the *anthropogenic and atmospheric impact* and the *soil-plant cycling model* in the year 1915, without
779 anthropogenic impact, without former atmospheric deposition and without soil-plant cycling. f_{Zn} values are illustrated with red, green and grey shading,
780 respectively, and the $\delta^{66}Zn_{soil}$ values are indicated with dotted horizontal lines.

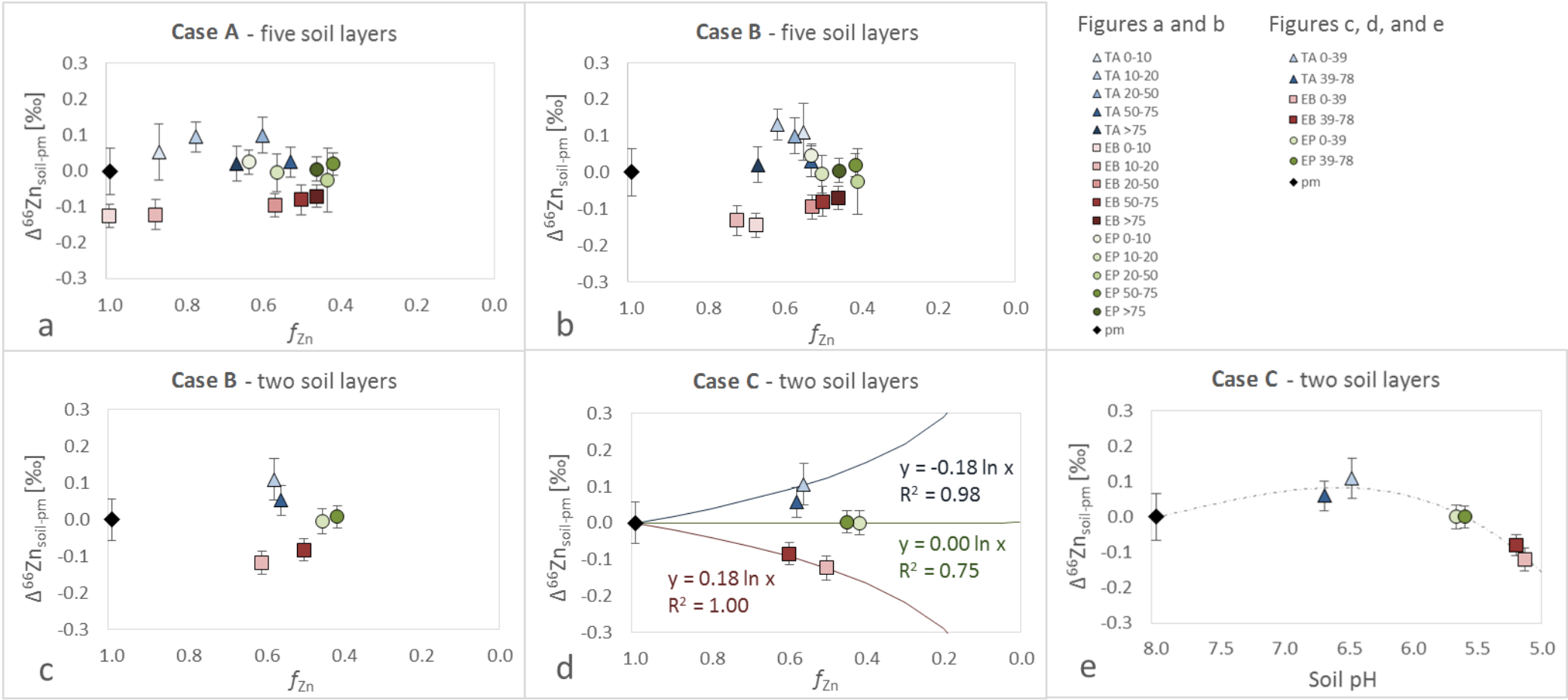

Figure 3. Relationship of the apparent isotope fractionation between soils and parent materials ($\Delta^{66}Zn_{soil\text{-}pm}$) with (a-d) the remaining Zn fraction in soils (f_{Zn} = τ_{Zn} values + 1) as well as (e) the soil pH, for Tänikon (▲), Ebikon (■) and Ependes (●). (a) **Case A**: current values in the year 2015 with anthropogenic impact, with former atmospheric deposition, and with soil-plant cycling. (b & c) **Case B**: values in the year 1915 without anthropogenic impact and former atmospheric inputs but with soil-plant cycling. (d and e) **Case C**: values in the year 1915 without anthropogenic impact and former atmospheric inputs and without soil-plant cycling. Rayleigh fractionation factors for soil formation in (d) were -0.18, -0.18, and 0.00 at Tänikon, Ebikon, and Ependes, respectively. Soil pH values in (e) were measured by the NABO in 1985[9] and the dotted line is for illustration purpose only and does not represent a model.