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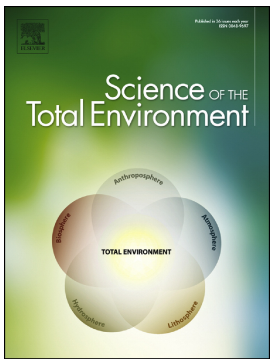

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Soil (microbial) disturbance affect the zinc isotope biogeochemistry but has little effect on plant zinc uptake

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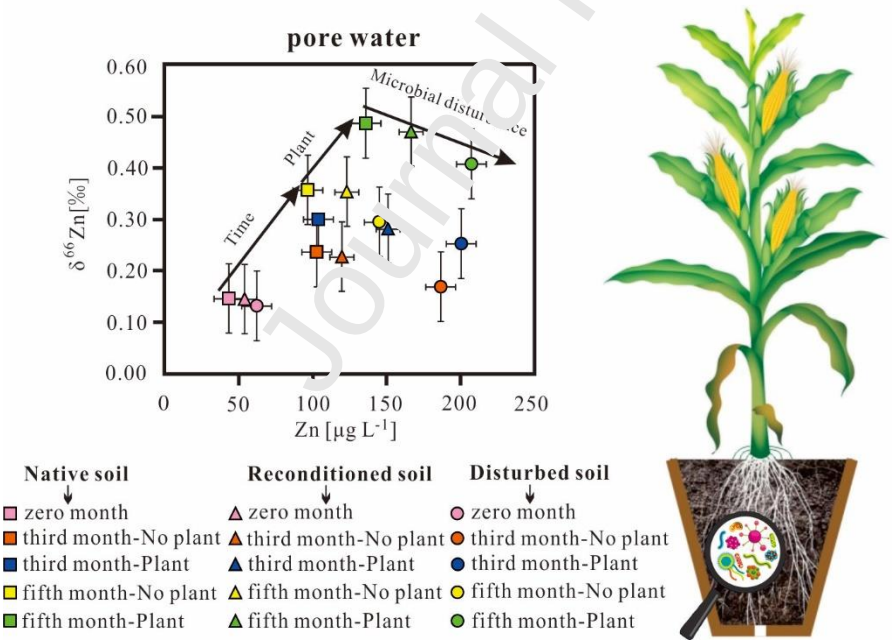
ABSTRACT: Zinc (Zn) is an important micronutrient but can be toxic at elevated concentrations. We conducted an experiment to test the effect of plant growth and soil microbial disturbance on Zn in soil and plants. Pots were prepared with and without maize and in an undisturbed soil, a soil that was disturbed by X-ray

sterilization and a soil that was sterilized but reconditioned with the original microbiome. The Zn concentration and isotope fractionation between the soil and the soil pore water increased with time, which is probably due to physical disturbance and fertilization. The presence of maize increased the Zn concentration and isotope fractionation in pore water. This was likely related to the uptake of light isotopes by plants and root exudates that solubilized heavy Zn from the soil. The sterilization disturbance increased the concentration of Zn in the pore water, because of abiotic and biotic changes. Despite a threefold increase in Zn concentration and changes in the Zn isotope composition in the pore water, the Zn content and isotope fractionation in the plant did not change. These results have implications for Zn mobility and uptake in crop plants and are relevant in terms of Zn nutrition.

Graphical Abstract



Highlights:

- Filling the pots and fertilization probably increased the Zn in the pore water
- The sterilization had biotic and abiotic effects that increased Zn release

- In the presence of plants the Zn concentrations in the pore water increase
- Maize plants regulate the Zn uptake and keep concentrations in the plant stable
- Zn isotopes indicate that Zn uptake mechanisms did not change despite doubled concentrations

Keywords: Zinc isotopes, trace metals, soil biogeochemistry, zinc deficiency, soil pollution, zinc nutrition

1. Introduction

Zinc (Zn) is an essential micronutrient for all biota, including plants (Shankar et al., 1998; Andreini et al., 2006; Maret, 2009). Adequate Zn supply, effective Zn absorption mechanisms and Zn redistribution in different plant organs are important factors affecting plant growth (Caldelas et al., 2011). Furthermore, the Zn content in crop-based food products has important implications for the human health (Bravin et al., 2012). The distribution of Zn in the soil-plant systems is partly controlled by the Zn concentration in the soil pore water, which varies with soil chemical properties such as the total Zn concentration in soils, soil pH, and soil organic matter (Mertens and Smolders, 2013). In addition, plants and soil microbes (soil bacteria and fungi) can secrete chelators and protons into the soil pore water or can change the soil redox conditions (Abou-Shanab et al., 2003; Ganguly et al., 2014). These microbially induced chemical changes in soils can be beneficial for the Zn nutrition of crops because it can increase the soil bioavailability of Zn in the soil and thereby also the soil-to-plant transfer of Zn (Hassan, 1996; Oosterousse et al., 1993; 2017; 2021).

Zinc isotope fractionation has been increasingly used to identify biogeochemical processes that control the transfer of Zn from soils to plants and within plants (Komárek et al., 2021; Wiggenhauser et al., 2022). For instance, the Zn isotope fractionation during Zn uptake and translocation varies with distinct Zn supply (Caldelas et al., 2011; Deng et al., 2013; Smolders et al., 2013; Weiss et al., 2021). This variation was attributed to the secretion of chelators that mobilize heavy Zn from the soil at low Zn supply (Smolders et al., 2013; Houben et al., 2014; Weiss et al., 2021) and distinct types of membrane proteins involved into root membrane

transport (Deng et al., 2016). Particularly, it is thought that the isotope fractionation during membrane transport may depend on Zn supply and Zn transport rates (Deng et al., 2016; Köbberich and Vance, 2018). Within plants, light Zn was more readily translocated from root-to-shoot and remobilized from vegetative to reproductive tissues (Caldelas et al., 2011; Arnold et al., 2015; Wiggenhauser et al., 2018). These observations were explained by the chelation of heavy Zn at excess supply and the retention of heavy Zn in the shoot through apoplastic absorption (Caldelas et al., 2011; Aucour et al., 2017; Wiggenhauser et al., 2018). These previous findings showed that Zn isotope fractionation in soil-plant systems can change with distinct Zn availability (i.e. soil properties). Hence, Zn isotope fractionation can be useful tool to detect changes of biogeochemical processes that control the Zn distribution in soil-plant systems in response to changing soil environments.

Although that several studies have shown that microorganisms can significantly fractionate Zn isotopes (Gélabert et al., 2006; John et al., 2007; Köbberich et al., 2018), potential effects of the soil microbiome on Zn isotope fractionation in soil-plant systems have not been determined yet (Caldelas et al., 2011). Fotios-Christos et al (2014) investigated the isotopic fractionation of Zn during surface adsorption and intracellular incorporation by representative Gram-positive (Bacillus subtilis) and Gram-negative (Pseudomonas mendocina and Escherichia coli) bacterial species and a natural bacterial consortium isolated from soil. The study found that *in vitro*, surface adsorption of Zn to bacteria, including natural bacterial consortium isolated from soils, favored heavy Zn isotopes at high Zn supply. The fractionation was ascribed to preferential complexation of heavy Zn to oxygen (O) donor atoms onto bacterial surfaces at high supply (Fotios-Christos et al., 2014; Tang et al., 2016). At low supply, light Zn isotope were adsorbed to the bacterial surfaces, likely because heavy Zn may have been complexed to organic ligands that were secreted by the bacteria into the nutrient solution (Jouvin et al., 2009; Marković et al., 2017). This change in Zn speciation in solution enriched the free Zn^{2+} pool in light isotopes and thereby also the Zn that sorbed to bacterial surfaces (Fotios-Christos et al., 2014). In addition,

bacteria took up heavy and light Zn, and dependent on the bacterial species (Fotios-Christos et al., 2014). Based on these *in vitro* results, we hypothesize that soil microorganisms and/or changes of soil the microbiome may influence the Zn isotope ratio in the soil pore water through Zn surface complexation, secretion of organic molecules, and through Zn uptake to maintain their metabolism.

The objectives of this study were to determine the influence of:

1) of the soil microbiome and the plant on Zn in the soil pore water

2) of the soil microbiome on the Zn distribution and Zn isotope fractionation within plants.

2. **Materials and methods**

More detailed information about material and methods can be found in the supplementary material.

2.1 Greenhouse experiment

The soil was sampled from the top 20 cm of an agricultural site in Frauenkappelen, Switzerland (Table S1). The soil has a high silt (53%) and sand (37%) content, a weakly acidic pH, a total Zn content of 67 mg kg^{-1} dry soil. The chosen soil represents a typical arable soil in Switzerland.

After sieving (10 mm), the soil was incubated in plastic boxes at a soil moisture of 60% water holding capacity (WHC) in the greenhouse for four weeks. The soil was then divided into a native soil (NS), disturbed soil (DS), and reconditioned soil (RS). DS and RS were sterilized by X-ray (at 25 kGy minimum to 60 kGy maximum (McNamara et al., 2003); Synergy Health Däniken AG, Switzerland). Microorganism were extracted from the untreated soil (SM 1.1) and added to RS after sterilization. All three soils were then incubated for one week before maize was sown.

The RS treatment was established to distinguish possible abiotic effects of sterilization from the microbial effects. The RS and DS treatment have been 'sterile' right after the X-ray treatment, but the DS soils likely

became recolonized by 'ambient glasshouse' and 'tap water' microbiome as the experiment was not performed under sterile conditions. Hence, the 'sterilized' treatment is defined as disturbance treatment. In addition, soil sterilization can cause abiotic effects besides biotic changes (Luo et al., 2022). Thus, the RS treatment was established in addition to the DS treatment. The abiotic sterilization effect was the same between DS and RS, while the microbial disturbance by soil sterilization was minimized in the RS treatment due to the reconditioning of microbial extracts. Therefore, it was assumed that the difference between RS and DS showed the microbial disturbance effect, and the difference between NS and RS reflected the abiotic sterilization effect. Thus the experimental design allows to differentiate between abiotic (NS vs. RS) as well as biotic effects (RS vs. DS).

The pots (7L) containing 6.5kg of wet soil (mean WHC 50%) were kept on saucers in a randomized manner in the greenhouse. The weekly fertilization in both NP and P pots started with 100 mL of 2 g L^{-1} complex fertilizer (Plantaktiv Starter 151, Hauert®) plus a 0.25 g of low iron ingredient (Sequestrene Rapid, Maag®), increasing to 200 mL complex fertilizer with a 0.5 g of high iron ingredient after one month. The complex fertilizer mainly contains 52% phosphate expressed as P_2O_5, 10% total nitrogen (8.4% NH_3-N and 1.4% NO_3-N), and 10% potassium expressed as K_2O). Three replicates with maize and three replicates without plants were grown for each treatment. In the beginning, the planted and unplanted soils were watered weekly by weighing pots and adjusting the WHC to 50%. From the third month of growth, plants were watered every second day as they needed more water for growth. The pore water was sampled with Rhizon samplers 0, 12, and 20 weeks after sowing (0.15 µm pore size, Rhizosphere Research Products BV, Netherlands). At week 12 and week 20 after sowing, soil samples were collected (SM 1.2). The maize was harvested after 20 weeks when cobs were well developed. Aerial plant parts were cut approximately 1 cm above soil surface and separated into stem, leaves and cobs (grains) prior to fresh weight determination. The roots were carefully separated from the

soil and washed by holding in a pot filled with MQ water and shaking the roots. The washing procedure was done twice (SM 1.5). All Plant samples were dried at 60 °C, dry weight was determined, and the plant parts were ground in a planetary mill equipped with agate beakers (RETSCH PM 200, Retsch GmbH, Germany).

2.2 Wet chemistry and analyses

Soil and plant samples were digested in a certified clean room (Picotrace, Göttingen, DE) on a hotplate. To this end, H_2O_2 (30%, trace select, Sigma Aldrich, St Louis, MI) and HF (48%, Suprapur, Merck, Darmsadt, DE), double distilled HNO_3 and HCl, and acid cleaned PFA beakers were used (SM 1.5). For isotope analyses, Zn was separated from the matrix by a one stage anion-exchange chromatography using an AG MP-1 Biorad® resin (BIO-RAD laboratories, Hercules, CA, USA) after a modified protocol from the method described by Maréchal et al. (SM 1.6). Zn concentrations were measured using inductively coupled plasma mass spectrometry (ICP-MS, 7700x, Agilent Technologies, Palo Alto, CA). Zn isotopes were measured using multiple collectors inductively coupled plasma mass spectrometry (Neptune Plus High Resolution MC-ICP-MS, Thermo Fisher Scientific, Waltham, MA). For instrumental mass bias correction, standard-sample bracketing and Cu doping was applied (SM 1.6). Three processing replicates of basalt, soil, and plant reference material were measured for Zn concentrations and Zn isotopes ratios (δ^{66}Zn; equation 1; Table S2). The Zn recovery upon the digestion of basalt, soil, and plant standards was 102 ± 13 % (Mean ± SD, n=11). Zn isotope ratio measurements of these reference materials were, within the external reproducibility of 2sd = 0.07 ‰, not distinguishable from isotope analyses conducted in other laboratories (i.e. the error bars of the individually processed and measured samples do not overlap). The range of δ^{66}Zn of the plant sample (ryegrass BCR-CRM281) of the interlaboratory comparison is large (0.08 to 0.50 ‰) while our measurements revealed a value of 0.26 ± 0.02 ‰ (n = 3 samples). Finally, repeat measurements of the isotope standard IRMM-3702 revealed a high accuracy and precision (0.01 ± 0.07 ‰).

2.3 Calculations and statistics

The isotope compositions of the samples are reported relative to IRMM-3702 Zn isotope standard using a notation according to equation 1:

$$\delta^{66}Zn = \left[\frac{(^{66}Zn/^{64}Zn)_{sample}}{(^{66}Zn/^{64}Zn)_{IRMM-3702}} - 1\right] \times 1000$$

The concentration values for whole plant or straw (stem + leaves) or shoot (straw + grain) samples were calculated according to equation 2:

$$Zn_{whole\ plant\ or\ straw\ or\ shoot} = \frac{\sum_i m_i c_i}{\sum m_i}$$

where *m* represents the mass of dry matter (DM, in g), *c* the Zn concentration (ng g^{-1}), and *i* the different plant parts of the whole plant (root + straw + grain) or of the straw (stem + leaves) or of the shoot (straw + grain). The δ^{66}Zn values for whole plant or straw (stem + leaves) or shoot (straw + grain) samples were calculated according to equation 3:

$$\delta^{66}Zn_{whole\ plant\ or\ straw\ or\ shoot} = \frac{\sum_i m_i c_i \delta^{66}Zn_i}{\sum m_i c_i}$$

where *m* represents the mass of dry matter (DM, in g), *c* the Zn concentration (ng g^{-1}), and *i* the different plant parts of the whole plant (root + straw + grain) or of the shoot (straw + grain).

The apparent isotopic fractionation between soil pools and/or plant parts was calculated according to equation 4:

$$\Delta^{66}Zn_{A\text{-}B} = \delta^{66}Zn_A - \delta^{66}Zn_B$$

where A and B denote the soil pools or the plant parts of interest in the soil-wheat system (e.g., root, straw, whole plant, and pore water extractable Zn) (Table S3, S4).

Significant differences of the mean of n = 3 experimental replicates were tested with ANOVA followed by a Tukey honestly significant difference (HSD) test. For all samples, equality of variances was given (determined using a Levene test and visual inspection), and the distribution of residuals was satisfactorily

normally distributed (visual inspection). For all tests, mean values were considered as significantly different from each other if the $p < 0.05$. Statistical tests and correlations were computed using the statistical software R (v.3.1.3; R Foundation, Vienna, Austria).

3. Results

3.1 Zn in pore water

The total soil Zn concentration of 66 mg kg^{-1} soil is in the normal range of non-contaminated arable soils (Table S1) (Mertens and Smolders, 2013). In addition, the weakly acidic soil pH (6.7) indicates that sufficient proportion of Zn in the soil was plant available. This is also indicated in the Zn pore water that was within a similar range (43-207 µg L^{-1}) than recently reported in non-contaminated agricultural soils (Table S3) (Blume et al., 2016; Imseng et al., 2018).

The Zn concentration and the Zn isotope fractionation in the soil-pore water increased during the experiment (Fig. 1). The Zn concentrations significantly differed between NS, RS and DS at sampling time points (Fig. 1), indicating that abiotic and biotic effects occurred through soil disturbance. The Zn concentrations in pore water increased with increasing soil disturbance (DS > RS > NS), with time, and were higher compared to soils without plants (except NS month 3). The δ^{66}Zn in the pore water did not differ between the soil disturbance treatments but shifted towards heavier isotopes over time of the experiment and in the presence of plants (Fig. 1).

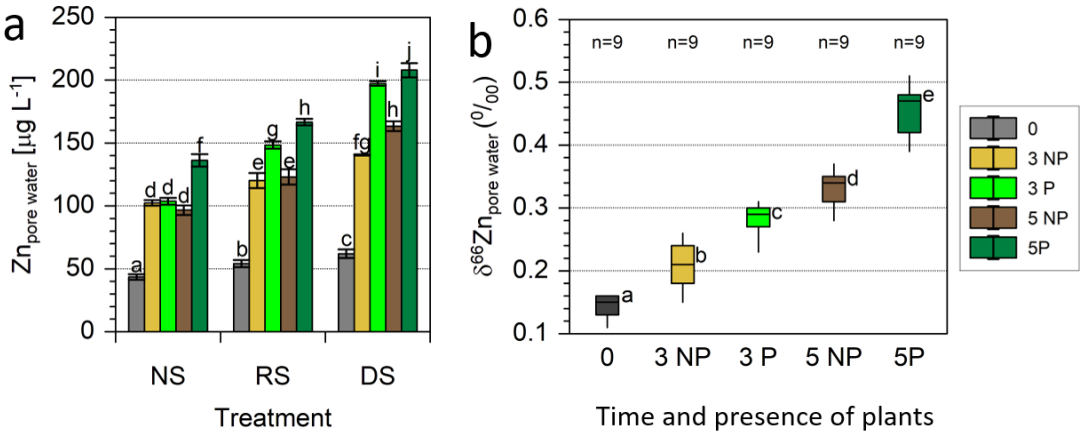


Fig. 1. a) Zn concentration and b) Zn isotope ratios in the soil pore water over time (3 = 3 months after sowing and 5 = 5 months after sowing). P (plant) denotes the presence of maize while NP (no plant) denotes the absence of maize. The abbreviations on the x-axes denote Native Soil (NS), Disturbed Soil (DS) and Reconditioned Soil (RS). In a) the error bars of the mean illustrate ±2sd of n = 3 experimental replicates. The boxplots in b) indicate the median, the lower and upper quantil (box) and the maximum and the minimum of the data (whiskers). Different letters beside the bars and boxplots indicate statisctically difference ($p < 0.05$) of the mean values.

3.2 Zn in the maize plants

Within the maize plant, Zn concentration ranged from 4.79 to 28.6 mg kg^{-1} DW and was lowest in the stem, followed by the roots and leaves, while the Zn concentration was highest in the grains (Table 1). The soil disturbance treatments had a strong effect on the biomass (NS > RS > DS) and the Zn concentration in maize (NS < RS < DS, Table 1). In contrast, the soil-to-plant transfer of Zn (i.e. the Zn mass in the plant) was not distinguishable among the treatments. In the grains, the Zn concentrations ranged from 18 to 29 mg kg^{-1} DW, indicating that the Zn supply was neither deficient nor excessive (Jouvin et al., 2009).

Light Zn isotopes were preferentially taken up by the plant ($\Delta^{66}Zn_{plant\text{-}pore.water}$ = -0.02 to -0.20 ‰, Table S5). Within the maize plants, about 90% of the Zn was transferred from the roots to the shoots while heavy Zn

isotopes were retained in the roots and light Zn isotopes were transported to the shoot ($\Delta^{66}Zn_{shoot\text{-}root}$ = -0.37 to -0.39 ‰). Within the shoot, 50 to 60% of the Zn was transported into the grains which were slightly enriched in light Zn isotopes compared to leaves and stems ($\Delta^{66}Zn_{grains\text{-}straw}$ = -0.09 ‰). However, soil disturbance had no effect on $\Delta^{66}Zn_{plant\text{-}pore.water}$, $\Delta^{66}Zn_{shoot\text{-}root}$ and $\Delta^{66}Zn_{grains\text{-}straw}$. Since the overall variability of the Zn distribution and Zn isotope fractionation within the plant was very low, no robust Rayleigh model could have been established (Fig. S2, S3).

Table 1. Maize biomass, Zn concentrations, Zn masses, mass fractions and $\delta^{66}Zn$ values in the different plant parts and the whole plants.

plant part	**treatment**[3]	**biomass**		**Zn concentration**		**Zn mass**		**$\delta^{66}Zn$**	
		[g]	sd[4]	[mg kg^{-1}]	sd[4]	[µg]	sd[4]	[‰]	sd[4]
roots	NS	4.15	±0.23 a	14.5	±1.37 b	60.3	±8.00 a	0.64	±0.01 a
	RS	3.60	±0.38 a	18.5	±2.19 b	66.8	±12.2 a	0.62	±0.01 a
	DS	1.48	±0.36 b	27.5	±3.20 a	40.2	±8.10 b	0.57	±0.01 b
stem	NS	23.4	±0.96 a	4.79	±1.31 b	113	±34.2 a	0.34	±0.02 a
	RS	22.3	±0.30 a	5.64	±0.66 b	126	±16.1 a	0.33	±0.02 a
	DS	17.1	±0.77 b	8.48	±0.80 a	145	±15.1 a	0.28	±0.02 b
leaves	NS	9.32	±0.40 a	12.8	±1.66 c	126	±19.0 a	0.28	±0.01 b
	RS	8.66	±0.45 a	18.1	±1.78 b	157	±21.3 a	0.26	±0.04 b
	DS	6.15	±0.13 b	24.8	±1.56 a	153	±12.7 a	0.21	±0.02 b
grains	NS	18.1	±0.69 a	18.0	±1.24 c	326	±34.5 a	0.22	±0.02 a
	RS	16.9	±0.67 a	21.9	±1.83 b	370	±17.4 a	0.20	±0.02 a
	DS	10.4	±0.75 b	28.6	±0.57 a	298	±15.4 a	0.16	±0.02 b
shoot[1]	NS	51.4	±0.23 a	11.0	±0.71 c	565	±36.0 a	0.25	±0.01 a
	RS	47.9	±0.62 b	13.6	±0.94 b	653	±47.8 a	0.23	±0.01 a
	DS	33.7	±0.88 c	17.7	±0.35 a	595	±16.1 a	0.20	±0.01 b

plant[2]	NS	55.5	±0.27 a	11.3	±0.66 c	625	±33.0 b	0.29	±0.01 a
	RS	51.5	±0.70 b	14.0	±0.81 b	719	±42.3 a	0.27	±0.01 a
	DS	35.2	±1.13 c	18.1	±0.44 a	635	±23.7 b	0.22	±0.01 b

[1]calculated with eq. 3-4 using stem, leaves, and grains

[2]calculated with eq. 3-4 using roots, stem, leaves, and grains

[3]NS=native soil, DS=disturbed soil, RS=reconditioned soil

[4]sd=standard deviation of the mean of n=3 experimental replicates. Letters indicate significant differences of the mean between treatments for the same plant organ

4. Discussion

4.1 The influence of time on the Zn concentrations and shift of Zn isotopes in the pore water

Our initial research questions targeted the effect of plants (research question 1) and microbiome (research question 2) on the Zn in soil solution. However, we found that the Zn concentrations in the soil pore water increased over time and became isotopically heavier independent of the presence of plants or the disturbance of the microbiome (Fig. 1). We think that the most probable reasons for the unexpected change over time are the sieving and the transfer of the soil to the pots. These processes mighthave changed the water household in the small open pots that were used for plant growth (higher evaporation, more watering) compared to the closed bigger boxes that were used to incubate the soil prior to irradiation (lower incubation, less watering) (Passioura and Viewpoint, 2006; Turner, 2019). The sieving and filling in the pots may have fragmented aggregates, changed the soil structure and increased the soil surface area, so that more Zn could be released to the pore water over the time of the experiment (Bronick and Lal, 2005; Ding et al., 2013). Thus, the filling of the soils into the pots disturbed the soil and caused a disequilibrium with the pore water. Over time, the soil equilibrated again and released more heavy Zn, which was originally adsorbed to the solid soil. Alternatively, the weekly fertilization might have caused a competition for the cation exchange sites (e.g. clay minerals, organic matter) in

the soil and Ca^{2+}, K^{+} or Fe^{2+} ions from the fertilizer might have replaced Zn and caused a release of Zn into the soil solution. We assume that one or a combination of the above-mentioned circumstances caused the increasing Zn concentrations and isotope ratios as an artifact of the experimental conditions.

4.2 The influence of growing maize on Zn concentration and isotopes in soil pore water

The presence of plants significantly increased the Zn concentrations in the soil pore water and caused a fractionation to more heavy Zn isotopes in the pore water (Fig. 1a). Plants can increase the release of cations such as Fe and Zn to soil pore water by lowering the pH in the rhizosphere and by secreting (chelating) organic molecules that strip Zn from the soil matrix (Mcgrath et al., 1996; Puschenreiter et al., 2017). In the presence of plants, the release of metal complexing ligands can increase the Zn fraction of (isotopically heavy) Zn complexes in the pore water (Arnold et al., 2010; Weiss et al., 2014). Hence, the higher Zn concentration and the heavier isotope composition in the pore water with plants suggest that plant root exudates solubilized heavy Zn isotopes in the soil. The maize plants tended to take up light Zn isotopes from the pore water, particularly after three and five months of plant growth (Table S5). Thus, the removal of light Zn isotopes from the pore water successively enriched the pore water in heavy Zn isotopes compared to the non-planted pots. Plants can take up heavy and light Zn isotopes from its Zn sources (Wiggenhauser et al., 2022). The uptake of light isotopes in our study could be related to the Zn speciation in the pore water or to root membrane transport (John et al., 2007; Markovic et al., 2016; Köbberich and Vance, 2018). The Zn species in soil solution can be divided into free, mostly isotopically light Zn ions and the Zn complexed to humic acids and root exudates with preferentially heavy isotopes (Jouvin et al., 2009). Therefore, the preferential uptake of light isotopes from the soil pore water suggests that maize took up free Zn from the pore water (Jouvin et al., 2009; Markovic et al., 2016). In addition, Zn membrane transport may have been kinetically controlled through comparably fast Zn uptake rates to cover the Zn demand of maize (John et al., 2007; Köbberich et al., 2018).

4.3 The influence of the biotic and abiotic sterilization effects on the Zn concentration and isotopes in soil pore water

The sterilization of a soil is known to affect not only the microbial community, but also the soil chemical properties, especially the N speciation (McNamara et al., 2003), iron reduction (Bank et al., 2008), conductivity, water-soluble carbon and DTPA-extractable Mn (Jiang et al., 2003). The changes in soil chemistry can induce the physiological response of a plant by increasing root growth or rhizoheath formation (Mahmood et al., 2014). Furthermore, sterilization by γ-rays can decrease Zn concentrations, increase soil pH, and increase DOC in pore water (Luo et al., 2001). After sterilization the soils can be rapidly recolonized but the biological community has a lower diversity and evenness than the native soil (McNamara et al., 2003; Marschner et al., 2004).

In our study, Zn concentrations in the pore water increased with soil disturbance (Fig. 1) which is in line with previous findings (McNamara et al., 2003). Reconditioning of the sterilized soils declined Zn concentrations in the soil pore water compared to the disturbed soil (Fig. 1),indicating that the microbiome had a significant effect (DS > RS) beyond the abiotic physicochemical effect of the sterilization. The difference in Zn concentrations between the NS, RS and DS treatments remained the same during the 5 months of soil incubation, even if total concentrations increased in all treatments. This result does not fully agree with findings that the soils can be rapidly recolonized after sterilization, which should also cause the differences between the soil disturbance treatments to decrease (Marschner et al., 2004). However, the constantly different concentrations in the pore water point on a pronounced biotic effect, if not on the microbial abundance than on the microbial community composition (Marschner et al., 2004).

Zn isotope ratios did not vary in pore water among the different treatments (Fig. 1). This result indicates that the increased release of Zn to the pore water with increasing disturbance did mobilize the Zn from a Zn pool with the same Zn isotope composition than the Zn in the porse water. The data further suggests that the Zn

desorption processes that release Zn into the pore water may be also the same among the different soil disturbance treatments. Hence, the distinct Zn isotope fractionation patterns that were observed for distinct bacterial species and communities *in vitro* (Kafantaris et al., 2013) cannot be necessarily extrapolated to complex soil-plant systems. Together, disturbance of the soil microbiome caused a biotic effect which changed the Zn concentration in the porewater, probably without changing Zn pools or release mechanism.

Comparing the different influences on the Zn concentrations and isotopes in the pore water it can be summarized that the temporal effect (physicochemical disturbance, microbial change) and the presence of plants had significant effect on Zn isotopes but minor effects on Zn concentrations, while the biotic and abiotic effects of soil disturbance by sterilization had no significant effects on Zn isotopes but strong effects on the Zn concentrations in the pore water (Fig. 2).

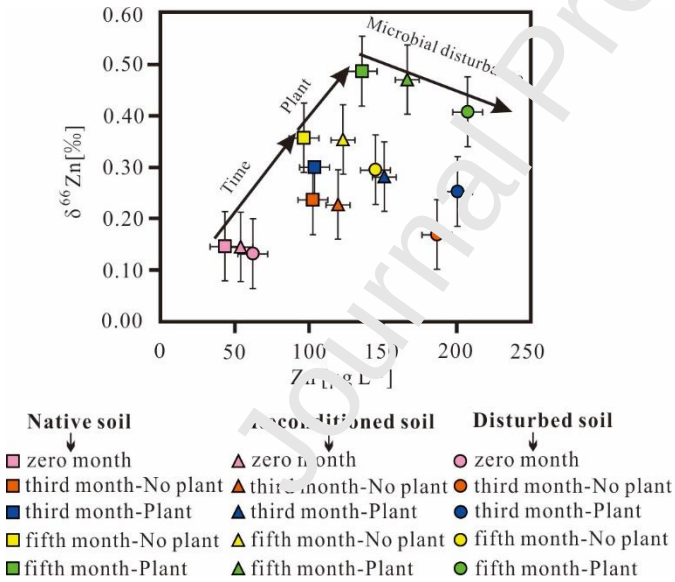


Fig. 2. Illustration of the different factors affecting the Zn concentration and isotope fractionation in the pore water. Pink color illustrates the beginning of the experiment, while orange color illustrates month three without plants, blue color month three with plants, yellow color month five without plants and green color month five with plants. Quadrants refer to undisturbed soil, triangles to reconditioned soils and circles refer to disturbed soils. The arrows illustrate the direction of the impact factors time, plant and microbial disturbance.

4.4 Mechanisms for Zn uptake and translocation did not change upon soil disturbance

Plants can adapt their nutrient uptake strategies as response to changing soil conditions (Wang et al., 2019, 2021). Zn uptake is metabolically mediated by two different transport systems: a high-affinity transport system, i.e., a protein carrier, that mainly operates at low Zn conditions and a low-affinity transport system, i.e., ion channels and electrogenic pumps, that is dominant under conditions with high Zn concentrations (Hacisalihoglu et al., 2001). The different transport systems can induce shifts in the plants Zn isotope compositions (Smolders et al., 2013; Weiss et al., 2021). The soil disturbance treatments of our study doubled the Zn concentration in the pore water in e.g., in the NS compared to the DS treatment (Fig. 1b). However, the Zn isotope fractionation during root Zn uptake ($\Delta^{66}Zn_{plant\text{-}pore.water}$) did not differ among the treatments (Table S3). These results strongly suggest that the Zn root uptake mechanisms including membrane transport (Deng et al., 2016; Köbberich and Vance, 2018) and secretion of chelating organic molecules into the rhizosphere (Smolders et al., 2013; Weiss et al., 2021) did not change upon soil disturbance. Hence, the change of soil conditions (i.e. Zn concentration) was not strong enough to change the root Zn uptake mechanisms.

The DW of the maize varied among the soil disturbance treatments (Table 1). For instance, the abiotic and biotic disturbance in the DS treatment reduced the root and shoot dry weight by 37% compared to the control treatment (NS). The same disturbance increased the Zn concentration by 61% while the Zn mass (i.e. the soil-to-plant transfer) and $\Delta^{66}Zn_{plant\text{-}pore.water}$ did not significantly vary (Table S5). This combination of changing DW and Zn concentration and equal Zn uptake refers to a so-called ‘concentration effect’ (Jarrell et al., 1981; McNamara et al., 2003). Previous studies showed that soil gamma irradiation can alter the plant available nutrient pools through cell lysis and damaging soil organic matter (McNamara et al., 2003). These alterations may have reduced the DW production of the maize plants. However, the soil-to-plant transfer of Zn did not

change and the Zn concentration in the plant tissues increased upon soil disturbance through a concentration effect (Table 1). As the maximum Zn tissue concentration is < 30mg kg^{-1} DW, Zn toxicity effects can be excluded as a cause of the reduced DW production. Alternatively, the irradiation can dissolve Mn-oxides and thereby increase the Mn concentration in the pore water (McNamara et al., 2003). The Mn concentrations in the leaves of the maize plants in the DS and RS ranged from 20 to 30 mg kg^{-1} DW which slightly above Mn deficiency thresholds (15 to 20 mg kg^{-1} DW) and should have no toxic effects (Schmidt et al., 2016; Wiggenhauser et al., 2018). Hence, it is not fully clear which factors reduced the maize biomass in the RS and DS treatments.

Changes in the soil environmental conditions such as water supply and nutritional status can alter the Zn translocation and remobilization pathways in plants (Waters et al., 2009; Etienne et al., 2018). In our study, the distribution of Zn and the Zn isotope fractionation within the plant did not vary upon soil disturbance (Table 1). These results suggest that soil disturbance was not strong enough to change Zn translocation and remobilization pathways in maize. However, Zn isotopes were systematically fractionated within plants as light Zn was preferentially translocated from root-to-shoot (Table 1). In addition, the maize grain was up to five times more concentrated in Zn and isotopically lighter than senescing stems and leaves. In wheat and rice, also light isotopes accumulated in reproductive organs which has been ascribed to the retention of heavy Zn isotopes in the apoplastic space while light isotopes were remobilized from senescent tissues via phloem to reproductive tissues (Arnold et al., 2015; Aucour et al., 2017; Wiggenhauser et al., 2018). Hence, the similar Zn isotope fractionation patters in wheat, rice, and maize suggest that similar processes control the remobilization of Zn in cereals.

5. Conclusion

Our results demonstrate that plants and soil microbiome as well as a physical disturbance or fertilization can

significantly influence Zn release from the soil to the pore water and thereby also the Zn isotope fractionation between the solid and the soil liquid phase. According to our findings, reduced mechanical and microbial disturbance decrease Zn in the soil pore water, but did not change plant uptake. In the opposite the maize plants themselves cause an increased Zn release to the soil pore water compared to unplanted soil, illustrating the plant nutrient mobilization mechanism. The findings highlight the effect of microorganisms, but also the ability of plants to mobilize Zn and to regulate Zn uptake at varying but moderate Zn concentrations in the pore water.

With respect to Zn isotope fractionation studies, we determined for the first time the role of soil microorganisms on Zn isotope fractionation in soil-plant systems and the possible post depositional isotope fractionation which is relevant for source tracing studies. Our study suggests that disturbance of the soil microbiome induces no significant effect on Zn isotope fractionation in complex soil-plant systems. Furthermore, soil microorganisms may not significantly contribute to post-depositional Zn isotope fractionation of anthropogenically introduced Zn. Instead, mechanical disturbance (e.g., ploughing), nutrient management (e.g., fertilization), and plant growth (e.g., root exudates) can alter the Zn isotope composition in soils through e.g., distinct Zn output through leaching. However, similar studies should be conducted in Zn limited and Zn polluted soil-plant systems to further test the impact of soil microorganisms Zn isotope fractionation in soil-plant systems.

Author Contributions

Xiaowen Liu: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - Original draft, Visualization. **Yi Huang**: Writing - Review and Editing, Project administration, Funding acquisition. **Hang Guan**: Conceptualization, Methodology, Investigation, Writing - Review and Editing, Project administration. **Matthias Wiggenhauser**: Validation, Formal analysis, Data curation, Writing - Review and Editing, Visualization, Supervision. **Veronica Caggìa**: Investigation, Writing - Review and Editing. **Klaus Schlaeppi**: Conceptualization, Resources, Writing - Review and Editing. **Adrien Mestrot**: Conceptualization, Methodology, Resources, funding acquisition, Writing - Review and Editing, Project administration. **Moritz Bigalke**:

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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