



# Can earthworms alleviate nutrient disorders of plants subjected to calcium carbonate excess?



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## ABSTRACT

Earthworms are recognized to affect soil nutrient cycling with important consequences for plant growth. Nutrient bioavailability is often limited in calcareous soils. Exactly how earthworms can impact availability of nutrients in higher plants grown on calcareous soils still remains an open question. The objectives of this study were to investigate in pot experiments complex nutrient dynamics of interactions between non-calcareous soil and earthworms with special emphasis on the availability of iron (Fe), zinc (Zn), manganese (Mn) and nitrogen (N) to plants (*Cucumis sativus* L. and *Zea mays* L.) subjected to calcium carbonate (CaCO<sub>3</sub>) excess. A combination approach was performed including analysis of nutrient mobility in casts/soil, micronutrient movement via xylem of plants and plant elemental analysis. Addition of CaCO<sub>3</sub> (5% DW) significantly lowered the concentrations of Zn and Mn in xylem sap and/or shoot (by 1.8–5.7 times) of both cucumber and maize in comparison with plants that had not received CaCO<sub>3</sub>. Bioavailability of Fe, however, was not affected by CaCO<sub>3</sub> treatment. Beneficial effects of earthworms— endogeic *Aporrectodea caliginosa* (Savigny) and anecic *Lumbricus terrestris* L., for plant nutrient acquisition include an increased mobility of all micronutrients (by 1.3–4.0 times) and N (by 1.5–27 times) in casts or soil either with or without CaCO<sub>3</sub> treatment. However, these earthworm species were generally not of major importance in improving Zn and Mn translocation from roots to shoot in cucumber and maize, which was strongly impaired in CaCO<sub>3</sub> applied soil. The results clearly demonstrated that earthworm capabilities to alleviate plant Zn and Mn deficiencies induced by CaCO<sub>3</sub> applications are limited.

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## 1. Introduction

Earthworms have been considered as one of the important faunal groups affecting soil fertility and nutrient cycling with important consequences for plant growth and ecology. Earthworm burrowing activity and their high consumption rates affect soil aggregation dynamics, microbial indices, organic matter transfer and humification (Al-Maliki and Scullion, 2013; Bityutskii et al., 2012b; Blouin et al., 2013; Cortez et al., 1989; Edwards and Bohlen, 1995; Huang et al., 2014; Lavelle et al., 2006). Soil passage through earthworms can alter mineralization rate of organic matter and availability of some plant nutrients within newly formed aggregates (casts). Many studies have shown a positive impact of earthworms on production of nutrient-rich casts: an increased content of nitrogen (N) (Amador and Görres, 2005; Bityutskii et al., 2007; Decaëns et al., 1999; Eisenhauer and

Scheu, 2008; Laossi et al., 2011), available phosphorus (P) (Suarez et al., 2004), potassium (K), calcium (Ca) and magnesium (Mg) (Adejuyigbe et al., 2006; Jouquet et al., 2008) and some microelements (Bityutskii et al., 2012a; Wen et al., 2004) has widely been recorded. Although it has been commonly accepted that earthworms benefit plant growth and productivity (Partsch et al., 2006; Scheu, 2003) direct evidence for the effect of earthworms on nutrient availability in soil is still inconsistent and even controversial (Bityutskii et al., 2012a; Clause et al., 2014). One accepted pathway by which earthworm stimulates plant growth is increased N availability (Van Groenigen et al., 2014). However, earthworms can induce an increase in plant production even in a soil excess of mineral nutrients, e.g. N (Arancon and Edwards, 2011; Laossi et al., 2009). Therefore, other mechanisms may also be responsible for the positive effect of earthworms on plant production (Blouin et al., 2013; Brown et al., 2004; Scheu, 2003).

Micronutrient bioavailability in soils is often limited, particularly in calcareous soils (Lindsay, 1995; Marschner, 1995). Calcium carbonate is a major component of these soils, which buffers soil

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solution pH at 7.5–8.5, due to a high bicarbonate concentration. Low solubility of microelements such as iron (Fe), zinc (Zn) or manganese (Mn) at moderate or high pH induces the micronutrient deficiency, which has been the most common nutrient disorders on calcareous soils—a limiting factor for crop production causing considerable yield losses of crops throughout the world (Alloway, 2008; Graham and James, 2003). Soils with excess of calcium carbonate cover about 30% of world's land surface (Vose, 1982). However, limited information is available on how earthworms affect availability and uptake of micronutrients in higher plants, taken into account that the plants show different susceptibility to soil factors, thereby inducing micronutrient deficiency. To overcome Fe deficiency, plants develop specific mechanisms (Strategy I and II). Strategy I plants (dicots and non-grass monocots) respond to Fe deficiency by releasing  $H^+$  and reductants (phenols) and enhancing ferric reduction activity in rhizosphere. Strategy II plants (other gramineous plants) secrete phytosiderophores—Fe-chelating substances that solubilize Fe in soils (Briat, 2008; Römheld, 1987; Römheld and Marschner, 1986; Schmidt, 1999). In contrast to mechanisms of Fe deficiency, little is known about specific mechanisms responsible for Zn or Mn deficiencies in plants.

The objectives of this study were: (1) to determine the effect of earthworms on the mobility of mineral nutrients (Fe, Zn, Mn and N) in casts/soil, and (2) to determine whether earthworm-triggered effects on nutrient mobility are likely to be important in the availability of the nutrients to plants. Our hypothesis is that calcium carbonate has a significant influence in the contribution of earthworms to regulation of nutrient bioavailability in soil.

## 2. Materials and methods

### 2.1. Experimental design

The experimental design included two experiments. First, we examined the mobility of elements in earthworm cast material/bioturbated soil (experiment I) then we examined the availability of elements to plants in soil bioturbated by earthworms (experiment II). To simulate condition of calcareous soil calcium carbonate treatment was applied to a non-calcareous soil. However, liming a moderately acid soil is not directly analogous to a soil that is naturally calcareous.

The experiment design included two levels of two independent treatments (+/– $CaCO_3$  and +/-earthworms). Each treatment combination was replicated four times. Prior to the experiments +/-earthworms, fresh soil used in the + $CaCO_3$  treatments was mixed with the  $CaCO_3$  (50 g kg<sup>-1</sup> dry soil). *Trifolium pretense* L. (dried shoots) was provided as a food for earthworms (10 g kg<sup>-1</sup>) and pre-incubated for 1 week prior to earthworm addition by adding distilled water to reach 60% water holding capacity (WHC). Non-earthworm controls received the same clover inputs.

Experiment II was performed in two stages. The first stage involved a pre-incubation period (for 4 weeks) in the presence of living earthworms. The impact of *Aporrectodea caliginosa* on plants was examined in 2012, whereas that of *Lumbricus terrestris*—in 2013. We expected that during the pre-incubation stage earthworm-induced modification in mobility of nutrients affecting their availability to plants. At second stage of experiment II, the earthworms were removed from the soil and then plants (cucumber and maize) were grown for 4 weeks.

### 2.2. Soil material

The soil (Anthri-Umbri-Endogleyic Luvisols) was collected from a fallow field near the Biological Research Institute of Saint Petersburg State University, Peterhof, Russia. The main chemical

characteristics of this soil were a pH of 5.1 (determined in a 1:2.5 soil–water solution), 2.38%  $C_{org}$ , 0.18% total N, and 31 and 103 mg kg<sup>-1</sup> of dry soil available P and K respectively (determined in a 1:5 soil–0.2N HCl solution) (Arinushkina, 1970).  $CaCO_3$  application increased pH of bulk soil by about 2.2 units.

### 2.3. Earthworms and their casts

We used endogeic earthworms *A. caliginosa* (Savigny) and anecic earthworms *L. terrestris* (L.), which are commonly found in grassland ecosystems. Mature earthworms with an individual biomass of  $0.4 \pm 0.1$  g (*A. caliginosa*) and  $3.5 \pm 0.9$  g (*L. terrestris*) were selected. In experiment I, the earthworm casts were collected as described in Bityutskii et al. (2012a). After voiding their guts on moist filter paper for 4 days, 12 individuals from a single species were added to each experimental soil and kept in the dark at room temperature (24 h). During the procedure the soil moisture content was maintained at 60% WHC. Subsequently the earthworms were rinsed with distilled water and placed into Petri dishes (12 specimens per dish) and stored in the dark for 24 h. The soil samples without earthworms (control) were also placed into Petri dishes in amounts equivalent to amounts of earthworm casts (determined previously). Three independent experiments I were performed with the same results.

During the pre-incubation stage of experiment II that focused on testing nutrient availability to plants, earthworms were kept in one liter plastic pot filled with 1 kg soil (dry weight) at room temperature in dark for 4 weeks. The moisture of soils was maintained at 60% WCH by daily watering. Prior to seed sowing, earthworms were removed from pre-incubated soils and the soils thoroughly hand mixed in each pot (irrespective of earthworm presence). By contrast to endogeic species (*A. caliginosa*), anecic species (*L. terrestris*) live in vertical burrows connected with soil surface (Bouché, 1977). However, the experimental setting seems suitable as a model to mimic the natural conditions of both endogeic (*A. caliginosa*) and anecic (*L. terrestris*) species because they both impact the drilosphere and thus nutrient availability to plant roots.

### 2.4. Plant material and growth conditions

After the pre-incubation stage of experiment II, three plants of cucumber (*Cucumis sativus* L., cv. Semcross) or maize (*Zea mays* L., cv. Uk23K1) were grown in each plastic pot filled with 1 kg soil (dry weight). These species were selected because they are model plants for Strategy I and Strategy II type of root responses to Fe deficiency respectively (Marschner, 1995). Plants were grown in a room at the temperature regime of  $24 \pm 2^\circ C$ :  $20 \pm 2^\circ C$  (light: dark) with a day/night regime of 16/8 h, and photon flux density of  $200 \mu mol m^{-2} s^{-1}$  at plant height. Because of the hot summer, the temperature during plant growth was 3–4 °C higher in 2013 (June–July) than in 2012 (June–July). During the entire experiment the plants were irrigated daily with distilled water to keep soil moisture of about 60% WHC.

### 2.5. Plant analysis

The xylem sap was collected by a micropipette for 1 h after the stems were cut 2 cm above root base and after discarding of the exudates obtained during the first few minutes.

Chlorophyll of the youngest fully expanded leaves sampled at the end of experiments was extracted using pure acetone and absorbance was measured at 662 (chlorophyll a) and 644 (chlorophyll b).

At harvest, shoots of plants were thoroughly washed with distilled water, dried at 70 °C for 48 h, and then weighed. Dry shoot

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