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Effects of titanium dioxide nanoparticles on soil microbial communities and wheat biomass

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ABSTRACT

Titanium dioxide nanoparticles (TiO_2 NPs) are the most produced NPs worldwide and have great potential to be utilized in agriculture as additives for plant protection products. However, concerns have been raised that some NPs may negatively affect crops and soil microbial communities, including beneficial microbes such as arbuscular mycorrhizal fungi. Here we tested two different TiO_2 NPs (P25, E171) and a bulk TiO_2 (particle size >100 nm) for their effects on the diversity and community composition of soil microorganisms. In addition we tested whether increasing concentrations of TiO_2 NPs had effects on wheat growth and yield. Microbial diversity was analyzed using Illumina Miseq paired-end sequencing of ribosomal markers (prokaryotic $16S_{V3V4}$ and fungal ITS2 of the ribosomal RNA operon). Application of TiO_2 NPs altered the detected prokaryotic but not fungal community structure. Prokaryotic community structure differed significantly between the three NP treatments and the control treatment without NP, although differences were smaller compared to those between the positive and the negative control. Specific microbial taxa responded positively or negatively to particular TiO_2 NP treatments and, thus, may be used as bio-indicators for TiO_2 NPs. No negative effects on wheat growth and on arbuscular mycorrhizal root colonization were detected, and no evidence for a dose-response relationship between wheat performance and TiO_2 NP concentration was found. Overall, these results reveal that prokaryotes are more sensitive than fungi to the TiO_2 NP treatments.

1. Introduction

Nanoparticles (NPs) are increasingly being used in electronics, composite materials, paints, cosmetics, food additives, and a wide range of other applications (Piccinno et al., 2012; Heiligtag and Niederberger, 2013). For instance, TiO_2 NPs reveal favorable properties, e.g. good covering power of pigments, UV-light attenuation and photocatalytic qualities. Nowadays TiO_2 NPs are manufactured worldwide with an estimated production of 88,000 t y^{-1} (Keller et al., 2013). Because of the substantial fabrication and usage of TiO_2 NP containing products, NPs get unintentionally released into the environment. For example in the US, approximately 760 t TiO_2 NPs y^{-1} are released into soils by application of sewage sludge (Gottschalk et al., 2009). Because of their properties as photocatalysts or UV protectors, TiO_2 NPs have also a potential to be used in plant protection products to enhance their effectiveness and reduce the application amounts or to decompose persistent compounds faster (Gogos et al., 2012; Kah et al., 2013). However, systematic application of such products would dramatically increase the estimated inputs of TiO_2 NPs to soils (Gogos et al., 2012). Soils have a geogenic background of TiO_2 on average of 0.5%, suggesting a certain evolutionary adaptation of soil organisms to TiO_2 (Scheffer et al., 2002). However, TiO_2 in its nano-scale form might affect

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Abbreviations

AMF	Arbuscular mycorrhizal fungi
ANOSIM	analysis of similarity
CAP	Canonical analysis of principal coordinates
NPs	Nanoparticles
PCO	Principal coordinate analysis
PERMANOVA	Permutational analysis of variance
PERMDISP	analysis of multivariate dispersion
TiO_2	titanium dioxide

soil organisms differently than the natural occurring TiO_2 in soils, and potentially affect ecosystem functioning at various trophic levels (Gardea-Torresdey et al., 2014). For instance, TiO_2 NP might cover the root or soil particle surface and inhibit growth and functioning of some micro-organisms or impair root colonization by beneficial soil microbes (e.g. mycorrhizal fungi or nitrogen fixing bacteria). Hence, there is a need to investigate potential non-target effects of TiO_2 NPs on soil organisms, plants, and plant-associated organisms.

Soil microorganisms conduct important ecosystem functions. For example they are important for soil carbon cycling, nitrogen fixation, and nutrient acquisition for plants (Carney and Matson, 2005; Hättenschwiler et al., 2005; van der Heijden et al., 2008). A key group of soil organisms that associate with two thirds of all terrestrial plants are arbuscular mycorrhizal fungi (AMF) of the phylum Glomeromycota (Smith and Read, 2008; Smith and Smith, 2011; van der Heijden et al., 2015). AMF acquire limiting nutrients, especially immobile nutrients, such as phosphorus, for plants and can enhance plant growth. Wheat, which is of particular importance for human nutrition, is one of the plant species that can benefit from a symbiosis with AMF (Pellegrino et al., 2015). Even though soil microorganisms play a crucial role in cropping systems, there are only a few studies that investigate the effects of TiO_2 NPs on soil microbial community structure, plants and the symbiosis between AMF and plants (Du et al., 2011; Ge et al., 2011; Song et al., 2013; Burke et al., 2014; Simonin and Richaume, 2015). For instance, soils planted with maize and soybean had been exposed for six weeks to 200 mg TiO_2 NPs kg^{-1} soil (Burke et al., 2014). While plant biomass and soil bacterial community structure were not affected by TiO_2 NPs, AMF communities were altered (Burke et al., 2014). In another study assessing AMF communities on soybean roots, no effects were found (Burke et al., 2015). Even less studies used high-throughput sequencing tools to investigate the effects of TiO_2 NPs on soil microbial communities. In one study using bar-coded pyrosequencing, Ge et al. (2012) observed that soil bacterial community structure was altered when treated with 0.5–2 mg TiO_2 NPs g^{-1} soil. However, that study only focused on bacteria and so far there is no study that simultaneously investigated effects of different TiO_2 NPs on bacteria, fungi, wheat and AMF root colonization in one experiment.

The current study was conducted to evaluate whether different concentrations and qualities (primary particle size and crystal structures) of TiO_2 NPs in agricultural soil affect (1) the diversity of soil prokaryotic and fungal communities, (2) root colonization by AMF and phosphorus uptake of wheat, and (3) the performance (yield) of wheat. For this purpose, we used industrially relevant TiO_2 NPs, i.e., P25 (anatase/rutile) and E171 (anatase), and a bulk anatase control (>100 nm) for E171. We assumed that the smallest NP, i.e. P25, would reveal the strongest effects on microorganism composition and plant growth, and that the effect size decreases with increasing particle size (E171 < bulk TiO_2). Different photoreactivity (ROS production) due to the different crystal structures (anatase and rutile) were assumed to have low influence, because in soils dark conditions prevail. Concentrations were chosen to represent soils with application of sewage sludge (Sun et al., 2014), application of NP containing agrochemicals (Gogos et al., 2012) and accidental spill.

2. Material and methods

2.1. Nanoparticles used

Two different TiO_2 NPs (P25 and E171) and bulk TiO_2 with increasing primary particle diameters were used. P25 (Sigma Aldrich, USA, Art. No. 718467) had the smallest primary particle diameter of 29 ± 9 nm, E171 (Hombitan FG, Sachtleben Pigments, Germany) had a diameter of 92 ± 31 nm, and bulk TiO_2 (Sigma Aldrich, USA, Art. No. 232033) had a diameter of 145 ± 46 nm (Gogos et al., 2016). The size of bulk TiO_2 is taller than the nano-range (>100 nm) and is used as a non-nano control for E171. The characterization of the used NPs and their fate in soil and plant uptake are presented in detail in the companion study of Gogos et al. (2016).

2.2. Soil substrate

Brown earth soil with a sandy loamy to loamy fine fraction was collected from an agricultural field near Agroscope, Institute for Sustainability Sciences, in Zurich, Switzerland (coordinates N47° 25′ 39.564" E8° 31′ 20.04") (Gogos, 2015). The soil was mixed with sand (50% v/v). Soil properties were described by Gogos et al. (2016) and were: pH 7.7, 86% sand, 6% silt, 7% clay, cation exchange capacity 6 mmol + kg^{-1}, and nutrient contents were 37.6 mg kg^{-1} phosphorus and 85.3 mg kg^{-1} potassium determined by ammonium acetate EDTA extraction (Stünzi, 2006).

2.3. Experimental design and NPs addition to the substrate

Wheat (*Triticum* ssp. var. Fiorina, spring wheat, 3 seedlings per pot) was grown in soil exposed to three TiO_2 NPs, P25 and E171 in three concentrations (1, 100, and 1000 mg kg^{-1} soil) as well as bulk TiO_2 (1000 mg kg^{-1} soil). A control treatment without NP addition and a positive control with $ZnSO_4 \cdot 7H_2O$ (1000 mg kg^{-1} soil, Sigma-Aldrich, Art. No., Z0251) addition was also included. We used $ZnSO_4 \cdot 7H_2O$ because it has been shown to affect wheat growth as well as soil microbial community structures (Frostegård et al., 1993, 1996; Warne et al., 2008; Rousk et al., 2012). These nine treatments were replicated 7 times, resulting in a total of 63 pots.

Three different amounts (0.03, 3, and 30 g) of each TiO_2 NP (E171, P25) and 30 g of bulk TiO_2 were added to 300 g soil substrate (50% v/v sand and soil) in a 500 ml Schott bottle and shaken in a powder mixer (Turbula T2F, Switzerland) for 30 min. In order to prepare the highest concentration of 30 g TiO_2 NPs, two bottles with 15 g NPs and 300 g substrate each were mixed. These pre-mixed soil-particle mixtures were then diluted in 30 kg sand-soil substrate in a cement mixer for 6 h. This was done separately for each concentration 1, 100, and 1000 mg kg^{-1} for TiO_2 NPs E171, P25, and of bulk TiO_2. Control substrate was treated as the spiked substrate but without adding NPs. Pots (15 cm diameter, 20 cm high, Fig. S1) were filled with a drainage layer of sand (520 g) at the bottom, and then covered with 3.3 kg spiked substrate per pot. The total titanium concentration in the soils was determined by X-ray fluorescence spectroscopy at the end of the experiment to verify the exposure concentrations as described by Gogos et al. (2016). Titanium concentrations of the control soil was on average