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Differential uptake of Ag nanoparticle forms by wheat across field soils

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Understanding the environmental risks of engineered silver nanoparticles (Ag NPs) is complicated by transformation processes which occur in soil, such as dissolution and sulphidation, generating diverse Ag forms, altering bioavailability and uptake by organisms. Despite this, integrated studies linking nanoparticle speciation to plant uptake in soil systems remain limited, and gaps persist in understanding Ag transformations as a function of soil type and their consequences for bioavailability. Using complementary techniques we compared the speciation (XAS and spICP-MS), bioavailability, and uptake of three Ag forms, two Ag NPs (Ag NP, Ag_2S NP) and $AgNO_3$ (all at 10 mg kg^{-1}), in wheat (*Triticum aestivum* L) grown in three different field soils over 27 days. Results showed that Ag forms were differentially bioavailable (Ag NPs $>/\approx$ $AgNO_3$ $>$ Ag_2S NP), and that soil type can have a pronounced influence on the extent of Ag uptake (approx. 7- to 15-fold variation for NPs across soils). Notably Ag_2S NPs, though considered "less mobile", can be more bioavailable to wheat than other Ag forms in some soil contexts. XAS speciation analysis revealed slower transformation dynamics in Woburn soil that may influence the overall pool of bioavailable Ag. While no single soil property fully explained the observed patterns, these findings indicate that intrinsic nanoparticle properties alone are not always sufficient to predict environmental behaviour in soil systems.

Environmental significance

Engineered nanomaterials such as silver nanoparticles (Ag NPs) are increasingly released into soils, yet predicting their environmental behaviour and bioavailability remains challenging. This study shows that both nanoparticle form and soil properties strongly influence plant uptake, and that environmentally transformed forms, such as sulfidised Ag_2S nanoparticles, can exhibit comparatively high bioavailability under certain soil conditions. These results highlight the importance of accounting for environmental transformations and soil type when assessing the fate and bioavailability of nanomaterials, with implications for improving the robustness of environmental exposure and risk assessments.

Introduction

Engineered silver nanoparticles (Ag NPs) have distinct physicochemical properties from bulk silver, which are exploited in various applications and consumer products. Agricultural soils are considered potential sinks for nanoparticles (NPs), where Ag NPs can enter terrestrial environments through the application of biosolids as soil conditioners. Once in the soil, the interactions and transformations of NPs are influenced by their own physicochemical properties, such as size and capping agent, as well as by environmental conditions and properties of the receiving soil.[1] These interactions and transformations of the NPs in soil will all influence fate and bioavailability.[2] While hydroponic studies allow systematic and mechanistic insights into the uptake and effects of Ag in plants,[3] they do not account for the complexities of soil environments. Thus, studies that advance our understanding of how soil type influences Ag NP ageing and uptake under natural soil conditions are needed to enhance NP risk assessment for terrestrial environments.

Interactions between Ag NPs and soil colloids, sorption to the soil solid phase, dissolution, and oxidation–reduction processes collectively and dynamically govern Ag NP fate and

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behaviour in soil,[4] and consequently their bioavailability and toxicity. These NP-soil interactions are complex, and the prevailing soil physicochemical properties can both enhance or reduce NP mobility and bioavailability. The mobility of Ag NP in soils is generally low, as the majority of Ag is quickly sorbed to the soil solid phase.[5,6] However, transport and retention of Ag NPs are strongly modulated by soil properties; for example, batch tests using natural soils have demonstrated positive correlations between Ag NP retention and increasing clay content,[5] higher cation exchange capacity[7] and organic matter,[8] as well as interactions with soil inorganic colloids that promote heteroaggregation.[9] In addition, the dissolution of Ag NPs, resulting in the release of Ag ions, can alter interactions with the soil matrices and influence Ag retention, mobility and bioavailability.[4,5,10] Lower pH promotes Ag NP dissolution,[11] while the addition of dissolved organic matter (DOM) can reduce dissolution,[11,12] although this stabilising effect of DOM can be DOM composition and Ag NP concentration dependent.[13] As a result, the processes affecting retention and mobility of Ag NPs are not static but evolve as the Ag NPs undergo transformations.[4,5,10] These interactions between Ag NPs and soil can also drive transformation processes, giving rise to a range of Ag speciation forms, with soil properties influencing the extent, timescale, and nature of Ag NP transformation pathways.[4,5,10] This varied transformation has subsequent consequences for the bioavailability and toxicity of Ag to soil organisms.[2] Under certain soil conditions, Ag NP dissolution can occur, generating Ag^+ ions that have been suggested to be more bioavailable to organisms than particulate Ag NPs.[14,15] Whereas, in environments where Ag NPs are deposited in biosolids, they have been found to transform predominantly to sulphidised forms (Ag_2S NP) *e.g.* ref. 16–19. In this reduced state, Ag_2S NPs are generally considered to be more stable, less mobile and as a result less bioavailable and toxic than pristine Ag NPs.[10,14,19–23,36] However, several studies have also shown Ag_2S NP can be phytoavailable, with Ag_2S attaching to the root surfaces forming secondary chemical forms of Ag_2S, detectable by techniques such as XAS (X-ray absorption spectroscopy) analysis.[3,15] Both the susceptibility of Ag NPs to environmental transformations and the resulting uncertain consequences for bioavailability reveal the importance of understanding how these transformations influence Ag NP speciation, bioavailability and fate in environmentally relevant soil conditions.[3,10,15]

This study aims to understand the speciation and bioavailability of different Ag forms (ionic, pristine, and transformed NP, Ag_2S) in natural soils with varying properties, and to assess bioaccumulation in wheat (*Triticum aestivum* L). We investigated the availability and bioaccumulation of two Ag NP forms: pristine 22 nm Ag NP (Ag NP), a more environmentally relevant 24 nm sulphidised Ag NP (Ag_2S NP) as well as soluble silver ($AgNO_3$), in three field soils with differing properties, including texture, pH, and organic matter content. Bioaccumulation and Ag effects were determined by measuring total Ag concentrations, biomass, and nutrient composition in plant tissues. In two soils with differing bioavailability profiles, we assessed the temporal changes in speciation of Ag in soils and plant roots using X-ray absorption spectroscopy (XAS) techniques and quantified particulate Ag in the plant tissue using single particle inductively coupled plasma mass spectrometry (spICP-MS). This information enabled us to consider how soil properties influence the availability of various Ag forms in soils and how these interacting factors affect bioaccumulation.

Materials and methods

Nanoparticle characterisation

The NPs used in this study were supplied by AppliedNano, Barcelona. Both NPs were re-dispersed in milli-Q water containing 1 mg mL^{-1} of 55-kDa PVP as a capping and stabilising agent. Ag NPs had an additional content of sodium citrate (5 mM) acting as stabilising and reducing agent to enhance chemical stability during storage. Physiochemical characterisations such as size and surface charge are described in Lahive *et al.*[24] Briefly, mean size distributions were confirmed by transmission electron microscopy (TEM) as 21.9 ± 3.6 nm for the Ag^0 polyvinylpyrrolidone (PVP) coated NPs (Ag NPs) and the Ag-sulphide PVP coated NPs (Ag_2S NP) as 23.5 ± 10.2 nm. Zeta potential measurements indicated surface charges of −39.0 ± 3.1 mV for the Ag NPs and −32.4 ± 0.6 mV for the Ag_2S NPs. $AgNO_3$ was used as the Ag salt (>99%, Sigma-Aldrich, Germany).

Experimental soils

Natural field soils with varying soil properties were collected from UK sites, an upland site used for animal grazing at Rhydtalog, Wales, UK (hereafter N. Wales; Histosol; OS Grid Reference SJ224564), a low land arable soil from Woburn, UK (hereafter Woburn; Butts Close plot, Cambic/Luvic Arenosol, OS Grid Reference SP947366) and Lufa 2.2 a standard experimental soil (Lufa, Agricultural Research Centre, Speyer, Germany). Woburn and N. Wales soils were collected to a depth of 25 cm, all soils were air dried and sieved to <2 mm before use. Water holding capacity (WHC) was determined for all soils (Table 1). Soil texture, pH, cation exchange capacity (CEC), and organic carbon are summarised in Table 1, with methods described by Norrfors *et al.*[25] Elemental composition and extractable cations are given in Tables S1 and S2, details of methods are given in SI.

Exposure treatments and soil spiking with Ag forms

Soils were spiked to a single nominal concentration of 10 mg Ag kg^{-1} for each Ag form. While exceeding predicted environmental concentrations, this exposure concentration was selected to enable mechanistic assessment by ensuring detectable concentrations across sample types, thereby enabling detailed temporal characterisation of Ag

Table 1 Soil texture, and summary of selected properties of the three experimental soils. For details on analysis and soil properties, see Norrfors *et al.*[25]

	Lufa 2.2	N. Wales	Woburn
Texture (% by weight)			
Sand (%)	81.7	49.6	83.4
Silt (%)	11.7	39.6	9.5
Clay (%)	6.6	10.9	7.3
Soil properties			
WHC[a]	42	96	32
Organic carbon (%)	1.53	9.63	0.61
pH[b]	6.49	4.77	6.41
CEC[c] (cmolc kg^{-1})	9.7	33.3	13.3

[a] Water holding capacity. [b] Water. [c] Cation exchange capacity.

transformation, speciation and plant uptake across all sampling points, soils, and Ag forms, and direct comparison across the analytical approaches. Concentrated stock suspensions and spiking suspensions were made in ultrapure water (Triple Red, Buckinghamshire, UK) for each Ag NP and $AgNO_3$. A 50 ml volume of the spiking suspension was added to 2 kg batches of dry soil. The soils were mixed until homogenous and additional deionised H_2O (dH_2O) added to reach 50–60% WHC. Two control treatments of wheat were also included for each soil; 1) unamended soil with no Ag added (blank), 2) NP carrier control, 5 mM sodium citrate, added at the same volume as the NP stock (citrate). These controls were mixed and wetted as for the Ag treatments. All soils were then incubated for seven days in the dark at 20 °C before planting, final measured Ag concentrations can be found in Table S7. During the experiment soil samples were collected and frozen for subsequent analyses at the end of this incubation period (t_0).

Exposure conditions, plant and soil sampling

Common winter wheat (*Triticum aestivum* L, KWS Dacanto, Denmark) was exposed under controlled greenhouse conditions (20 °C ± 10 °C, 16 h light > 400 μmol m^{-2} s^{-1}, 70% ± 20% relative humidity), and monitored twice daily, with emergence rates following OECD 208 guidelines (>80%). More detailed information on the exposures can be found in the SI. Briefly, three replicate samples for each treatment were collected for analysis at 1, 4, 6, 13, and 27 days post-emergence. On day 1 (t_1) post emergence, five seedlings per replicate were pooled for analysis and individual seedlings were transferred to either small growing units (129.3 g DW) for day 4 (t_4) collection or larger growing units[24] (176.3 g DW) for 6, 13, and 27 day collections (t_6, t_{13}, t_{27}). Plants were watered every 2–3 days with deionised water (dH_2O), maintaining soil moisture content between 50% and 60% WHC. At each sampling point, plants were carefully removed, roots and shoots separated, cleaned in dH_2O, and dried at 70 °C to constant weight. Final dry weights were recorded. Soil from each unit was mixed and sampled for total Ag content, XAS analysis, and porewater collection. Soil water content was determined by drying a 7 g sample to constant weight at 70 °C.

Additional exposures were set up to ensure sufficient plant tissue for X-ray Absorption Fine Structure (XAFS) and spICP-MS analyses, with wheat plants grown in 600 g of spiked soil for two weeks post-emergence (t_{14}) in plastic cups under identical conditions but with wicks used to maintain soil moisture. Soil samples were snap-frozen and freeze-dried before XAS at the Ag K-edge. Plant tissues were collected as described, with additional 0.1 M EDTA and dH_2O washing steps before being snap-frozen and freeze-dried. Dried tissues were pooled, homogenised, and compressed into pellets for synchrotron analysis.

Porewater extractions

Soil porewater samples were collected as described in Lahive *et al.*[24] Briefly, 34 g wet weight of soil was collected from each replicate at t_1, t_6, and t_{27}, and dH_2O added to reach 100% WHC. The saturated soils were gently mixed and incubated for 24 h at 20 °C. Porewater was extracted from the saturated soil by centrifugation at 2000*g* (1 h at 4 °C) through 0.45 um polyvinylidene fluoride (PVDF) filters (Thames Restek, U. K.) pre-soaked with 0.1 M $CuSO_4$ (Sigma Aldrich, Germany). An aliquot of porewater filtrate was ultra-filtered using $CuSO_4$ primed 10 kDa polyethersulfone (PES) filters (Pierce™ Protein concentrators, Thermo-scientific, USA) centrifuged for 30 min at 4000*g*.[24] The porewater and ultra-filtrate (UF) were acidified at a ratio of 3 : 1 v/v filtrate to *aqua regia* (3 : 1 HCl (≥37%) and HNO_3 (≥69%), both TraceSELECT Honeywell Fluka, USA) and stored in the dark at 4 °C until analysis.

Silver analysis of plant tissues, soil and porewater

Total Ag analyses of the (digested) plant tissues, soils and porewaters were conducted using Flame Atomic Absorption Spectroscopy (F-AAS, Perkin Elmer AAnalyst 100) or graphite furnace (GF-AAS, Perkin Elmer PinAAcle 900Z) as described in Lahive *et al.*[24] Certified reference material (DOLT-4) was used in each run to ensure quality assurance for the analysis.

XAS analysis of Ag speciation

X-ray Absorption Near Edge Structure (XANES) spectroscopy of soil samples were conducted at XAS Beamline of the Australian Synchrotron (AS). The Extended XAFS (EXAFS) analysis of soil and root samples were examined at Beamline B18 of Diamond Light Source (DLS), UK at the Ag K-edge (25 514 eV). All spectra were acquired in fluorescence using a solid-state Ge detector (9-element at DLS, 100-element at AS), the soil spectra were acquired at room temperature, and the root samples using a cryostat at liquid N_2 temperature (*ca.*77 K). EXAFS spectra were acquired from −200 to +500 eV of the absorption edge at 0.5 eV step size, corresponding to *ca.* k = 11 in photoelectron wavenumber. Each spectrum

required 5 minutes and 10–60 replicate scans were averaged depending on the Ag content (3–200 mg kg^{-1}) to produce the final spectrum. Linear combination fitting (LCF) analysis of XANES spectra (E_0 − 30 − E_0 + 70 eV) analysis was performed using Athena software[26] to estimate the speciation as a combination of Ag_2S, metallic (Ag^0) Ag-NPs, Ag_2S NPs, AgCl, $AgNO_3$, Ag_3PO_4, Ag-oxalate, Ag_2CO_3, Ag_20, Ag bound to thiol (Ag-SH, cysteine), ferrihydrite (Ag-Fh) and natural organic matter as Suwannee River humic acids (Ag-HA, HA = SRHA standard II, IHSS) (Fig. S1). Quality of the LCF models was evaluated using the R-factor output from Athena,[26] where lower values indicate a better agreement between the measured and fitted spectra. EXAFS was used to distinguish between the inorganic sulphide (Ag_2S or Ag_2S NP) from Ag bound to organic sulphur (Ag-HA) using the extended region k = 3–8 $Å^{-1}$.

Particulate silver analysis in plant tissues using spICP-MS

The particulate fraction of silver in wheat root and shoot tissues was analysed using single-particle inductively coupled plasma mass spectrometry (spICP-MS). Sample preparation followed the protocol described by Clark *et al.*,[27] with minor modifications. Full details of the extraction, calibration, and data processing are provided in the SI methods. Briefly, freeze-dried tissues were subsampled (10–25 mg), digested in a tetramethylammonium hydroxide (TMAH) and $CaCl_2$ solution, and left overnight at room temperature. Samples were subsequently diluted and analysed using an iCAP TQ ICP-MS (Thermo Fisher Scientific) operated in kinetic energy discrimination mode. Instrument calibration, tuning, and performance checks were conducted according to manufacturer specifications. A 3 ms dwell time and 60 s acquisition time were used for each sample, targeting the ^{107}Ag isotope. Transport efficiency was determined daily using a 60 nm Au NP standard, and sample uptake rate was measured gravimetrically. Particle mass concentrations were calculated from approximately 20 000 data points per sample and normalised to dry tissue weight. The particulate fraction was expressed as a percentage of total Ag concentration.

Elemental analysis of wheat tissue

A total of 5–20 mg of the shoot or root from each respective treatment was weighted into a glass vial. Two ml of nitric acid (primar plus grade) was then added and left to digest at room temperature for 1 h. Following this, the vials were placed on a hot plate and the temperature increased to keep the acid simmering until the digestion was complete (the absence of vapour and a clear liquid). Then the samples were left to cool and transferred to a 15 ml centrifuge tube and diluted to 10 ml using ultrapure water and spiked with indium and iridium to give a final concentration of 5 μg L^{-1} to act as an internal standard for analysis. The samples were then analysed for total Ag concentrations using ICP-MS and for the electrolyte (Na, Mg, K, Ca, Cu, Zn and Fe) concentration using ICP-OES. Procedural blanks were also included to ensure no contamination from the digestion process. Samples were compared to standards of a known concentration for the elements of interest, and the standards were matrix matched.

Data analysis

Normality was verified (Shapiro Wilk test), and data were log-transformed where necessary. One and two-way ANOVAs were performed to examine the effect of Ag treatment and soil type on Ag content in porewater or plant tissues at each sampling time point. Tukey HSD (honest significant difference) post hoc tests were used to determine significance ($p < 0.05$). All statistical analyses were conducted in R[28] (v.3.6.2.) and visualised in ggplot2[29] and ggpubr.[30] Outliers were confirmed using studentized residuals with Bonferroni correction (R package: *car*[31] v3.0–8. $P < 0.05$). Enrichment factor of Ag (AgEF) is defined as the ratio of Ag concentration in plant tissue from plants grown on Ag-treated soil to that in plant tissues from control soils. The bioaccumulation factor (BAF) is defined as the ratio of Ag concentration in plant tissue to the Ag concentration in the soil in which the plant was grown.

Results and discussion

Phytoavailability and translocation of Ag is soil and Ag NP form-dependent

Tissue Ag measurements revealed a clear influence of soil type on Ag accumulation in wheat. Consistent differences between soil types were observed across multiple Ag forms (Fig. 1A). By the end of the exposure (t_{27}) bioaccumulation of all Ag forms was significantly greater (p = 0.00) in Woburn soil compared to the other two soils. Root Ag concentrations were 7-fold higher for Ag_2S NPs, and up to 15 and 14-fold for Ag NP and $AgNO_3$, respectively (Fig. 1A, Table S4). There was little difference in Ag bioaccumulation between plants grown in the Lufa and N. Wales soils. Despite similarities in uptake in Lufa and N. Wales, soils exhibited contrasting properties, including texture (*e.g.*, 81% sand in Lufa *vs.* 49% in N. Wales), pH and % OC (Tables 1, S1 and S2). Lufa and Woburn soils were more comparable in texture and key properties, although differing in their % OC and CEC, indicating that despite similarities in uptake patterns between Lufa and N. Wales, there was no clear relationship with these specific soil properties. Bioavailability is unlikely to be governed by a single property, instead, interactions among soil characteristics, including texture, OC content, pH and mineral composition, are likely to collectively influence Ag retention, transformation, mobility, and plant uptake.

Plants take up nutrients from the soil porewater, so solutes, including Ag and NPs, in this phase are considered mobile and more available for uptake. Porewater measurements at several time points during the exposure showed that Ag concentrations were both soil and Ag form dependent (Fig. S3 and Table S5). Total Ag concentrations (porewater) were generally low across all Ag forms (<1.2% of

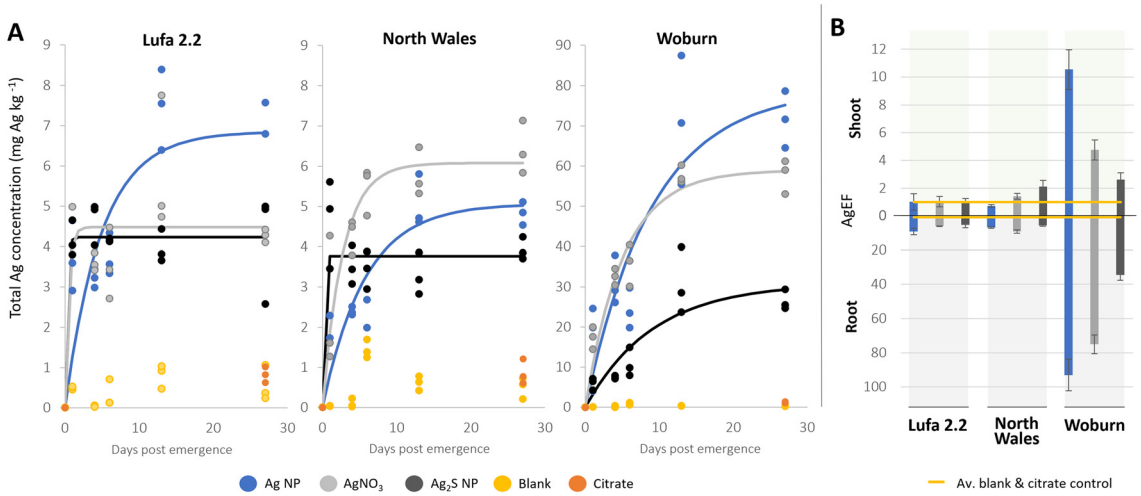

Fig. 1 A. Ag concentration in wheat root exposed to different Ag forms. Each dot represents an individual replicate and dot colours indicates the Ag form to which each was exposed. Lines represent uptake rates for Ag derived from one-compartment models (Lahive *et al.*[24]) B. Silver enrichment factors of roots and shoots exposed to Ag forms at t_{27}. Top bars show AgEF for wheat shoots and below bars show AgEFs for the roots. Yellow line shows the mean AgEF of wheat grown in both control soils.

total Ag), in line with other Ag soil studies,[13,22] likely reflecting adsorption of the negatively charged NPs (zeta potential <−30 mV) to positively charged soil surfaces.[5] Porewater Ag concentrations were generally higher in Woburn soil compared to Lufa and N. Wales soils, although these differences were small and did not clearly reflect plant uptake patterns (Fig. S3 and Table S5). Ultra-filtered (UF) Ag concentrations were lower still, with few significant differences between treatments, suggesting limited free dissolved Ag across all soils.

Comparable root Ag concentrations to those measured in Lufa and N. Wales soil were observed in previous studies with Lufa soil[24] and similar sandy loam soil.[32] Yang *et al.*[32] reported wheat root AgEF of ~3 after 3 months, compared at t_{27} in this study to an AgEF of ~9 and 7 in Lufa and N. Wales soils, respectively, (Fig. 1B). The duration of exposure could explain the differences, as our temporal analysis found root Ag concentrations plateau and AgEFs decrease over time (Fig. 1B), likely due to growth dilution as the root biomass increases.[24] Biomass data (Table S4 and S8) indicate that the substantially higher Ag concentrations measured in wheat exposed in Woburn soil were not accompanied by comparable effects on root or shoot growth at t_{27}. The similarity in the Ag NP AgEFs between Lufa and N. Wales highlights the distinctively high AgEFs in Woburn soil (34, 75 and 93 in Ag_2S NP, $AgNO_3$ and Ag NP respectively, Fig. 1B). Ag_2S NPs are generally associated with lower Ag uptake compared to other Ag NP forms.[3,10,14,20,21,23] Although this pattern was not clearly identified in Lufa and N. Wales soils, we did observe significantly lower ($p < 0.001$) Ag accumulation in wheat roots exposed to Ag_2S NP in Woburn soil compared to other Ag forms in the same soil (Fig. 1B).

Consistent with other studies,[23,32,33] the majority of Ag was found in the roots of exposed wheat. Translocation of Ag to the shoots was low across all treatments and soils (Fig. 1B, Table S4), in line with previous reports showing limited heavy metal translocation in wheat and rice.[34,35] Higher Ag concentrations were found in the roots of wheat exposed in Woburn soil, with correspondingly higher Ag concentrations also observed in the shoots compared to the other soils (Table S4). However, this higher shoot concentration is not directly proportional to that found in the roots, resulting in a lower root to shoot ratio for Woburn.

Consistent with previous studies,[3,33,44,45] Ag from Ag_2S NP was bioavailable to wheat and was internalised and translocated to the shoots under various soil conditions (Fig. 1B, Table S6). However, the extent of uptake varied substantially between soils, highlighting the importance of soil in controlling Ag_2S NP bioavailability. Ag uptake from Ag_2S NPs in Woburn soil exceeded that observed for all other Ag forms in Lufa and N. Wales soils (Fig. 1). Wheat exposed to Ag_2S NP in N. Wales soil, where shoot concentrations were almost equivalent to those observed in the Woburn soil (Fig. 1B). This increase of shoot Ag in the N. Wales Ag_2S NP exposure is likely due to the higher exposure Ag concentration in the soil for this treatment (see measured soil concentrations in Table S3). Interestingly, this Ag enrichment in shoots did not coincide with an equally elevated root Ag concentration compared to other Ag forms in N. Wales soil and was significantly less than root Ag concentration in Woburn. This suggests that Ag transfer to shoots is not solely dependent on root concentration and that shoot translocation of Ag cannot always be predicted by root Ag concentrations alone. Though there was some variation between soils, the porewater Ag concentrations were a small percentage of the total Ag throughout the experiment, and the differences between soils and Ag

forms do not reflect the uptake patterns seen between Ag concentrations in the roots across soils (Fig. S3 and Table S5).

Ag form influenced plant biomass but not nutrient composition

Ag NP exposure has been shown to disrupt wheat growth in sand, reducing root and shoot length in a dose-dependent manner.[37] In this study, significant differences in biomass were found between Ag treatments, indicating opposing biological effects of Ag forms. Notably, root biomass in plants exposed to $AgNO_3$ in Woburn soil was significantly higher ($p < 0.034$) compared to both controls (Fig. S2). For shoot biomass at t_{27}, significant differences were observed only between Ag NP and Ag_2S NP in Woburn soil, with Ag NP-exposed plants showing reduced biomass ($p = 0.03$). NPs, including Ag NPs, can alter micro- and macronutrient content in various plants.[38–40] Measurements of nutrients in the plants grown in Lufa and Woburn soils revealed significant differences between Ag treatments and the blank controls, indicating possible alterations in plant metabolism, even at low Ag concentrations in the roots. For example, increased Na concentrations were observed in the roots exposed to Ag_2S NP in Woburn soil, while Mg and Fe uptake concentrations were reduced in roots exposed to Ag NPs (Table S7). Given the greater Ag concentrations in roots and shoots of wheat in Woburn soil, greater disturbances in nutrient composition might have been expected. However, we see no clear pattern in the disturbance in nutrient composition within or across soils, suggesting no definitive change in nutrient status in plants grown in Ag spiked soil, although longer-term effects cannot be ruled out.

Speciation in soil is Ag form, soil type and time dependent

To determine whether differences in Ag concentrations in plants grown in Woburn and Lufa soils resulted from variations in transformation processes of Ag forms, we used XAS to analyse the temporal transformation of Ag forms in both soils and root tissues. XANES analysis revealed that Ag transformations were soil dependent (Fig. 2). In both soils, Ag NP and $AgNO_3$ transformed more quickly after the initial spiking (Fig. 2, fresh), producing similar secondary Ag species (Ag-humic acids, Ag-ferrihydrite, and Ag-thiol), while Ag_2S NP showed little transformation. The presence of an Ag^0 component in the $AgNO_3$ samples in both soils suggests partial reduction of Ag^+, potentially through interactions with soil organic matter such as HA, which have previously been shown to reduce Ag^+ to nanoparticulate/metallic forms.[53] In Lufa soil, ~50% of Ag from Ag NP had reacted within 30 minutes of spiking (Fig. 2, fresh), and by the end of the experiment (t_{27}), only ~12% remained as pristine Ag NPs. In contrast, Ag NPs in Woburn soil showed slower transformation, decreasing from 72% (fresh) to 28% at t_{27}. Linear combination fitting (LCF) analysis indicated that Ag from Ag NPs and $AgNO_3$ became complexed with thiol ligands, which comprised the dominant fraction of secondary species in both soils by t_{27}. This similarity in speciation could indicate the dissolution of Ag NP followed by rapid sorption of released Ag^+ to thiol ligands. However, the slower rate of transformation of Ag NPs suggests that soil properties influence the formation of secondary species. Iron oxides such as goethite and hematite can inhibit Ag NP dissolution by 25–75% and 3–68%, respectively.[41] Woburn soil contains higher goethite and Fe content (3 and 9 times more respectively, see Table S1), which may reduce the formation of secondary species through heteroaggregation and surface interactions with Fe oxides. In addition, Woburn soil's low organic matter (OM) content may help explain the slower Ag NP transformation, as OM can limit the availability of dissolved organic ligands that promote Ag^+ complexation,

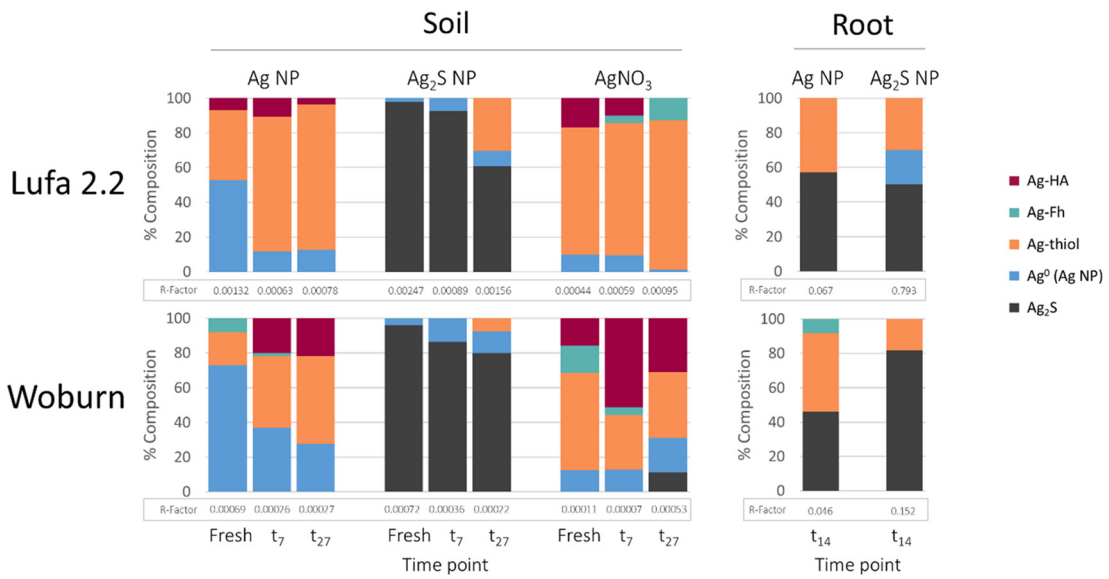

Fig. 2 Percentage composition determined by LCF analysis of Ag XANES (soil) and EXAFs (root) spectra showing percentage of Ag speciation in soils spiked with Ag forms at 3 timepoints. Fresh-freshly spiked soil, reading was taken at approx. 30 min. Percentage of Ag_2S containing standards are combined to give Ag_2S. R-factor (goodness-of-fit statistic) obtained from linear combination fitting of XAS spectra.

slowing the formation of secondary species and allowing a greater proportion of Ag NPs to electrostatically interact with mineral surfaces.[25] Such interactions in turn may limit Ag^+ release and mobility, while promoting retention of both particulate and dissolved Ag in the rhizosphere, potentially enhancing plant uptake. However, only minor evidence of Fe–Ag interactions was observed in the XAS data, suggesting that Fe content is not the main property driver for increased Ag uptake in plants grown in Woburn soil. Although it should be noted that weaker electrostatic associations, while potentially important, may fall below the minimum resolvable fraction of 5–10% for XAS where XAS-derived speciation estimates are dependent on the uniqueness of reference standards included in the linear combination fitting analysis.

Consistent with their known environmental stability[14] the majority of Ag in Ag_2S NP remained bound to inorganic S throughout the experiment, although there was evidence of secondary Ag species being formed in both soils. Lufa soil showed greater dissolution of Ag_2S NP, where LCF revealed that ~30% of Ag was complexed with organic S (thiol) by t_{27}, compared to less than 10% in Woburn soil (Fig. 2). The increased thiolation of Ag from Ag_2S NPs in Lufa soil occurs during the experimental period, as the soil becomes more impacted by growing plant roots, and may reflect a soil-specific difference in rhizosphere composition.[42] The slow reactivity of NPs in Woburn soil could indicate that the NP forms remained intact longer. Uptake of intact NPs has been reported to cause damage/architectural changes to the root,[3,43,44] which could help explain the increased bioavailability of Ag if more NP are associating with the roots in Woburn soil. It is also worth noting that the amount of mobile (porewater associated) Ag in both soils was low (generally <1% of total Ag added) and bulk XAS analysis cannot differentiate the speciation occurring specifically in the mobile fraction, which is likely most accessible to wheat. The low level of Ag in the porewater relative to the bulk soil suggests that transformations are more likely governed by processes occurring at the soil-Ag interface or within the rhizosphere, which are not resolved in the bulk soil analysis. Further insight into these processes is therefore considered through analysis of Ag speciation in the wheat root tissues.

We used the EXAFS region of the Ag XAS spectra to compare the speciation in wheat roots following exposure to Ag NPs and Ag_2S NPs in both Lufa and Woburn soils. EXAFS revealed that, consistent with the soil analysis, Ag in roots of Ag_2S NP exposed wheat in Woburn soils remained predominantly Ag_2S bound (Fig. 2), aligning with previous studies exposing Ag_2S NPs to wheat, cowpeas, and cucumber.[44,45] The formation of Ag_2S in Ag NP exposures meant that ultimately the EXAFS analysis of the roots generally revealed similar Ag speciation profiles between the Ag NP forms (Fig. 2 - Root). Despite this general similarity, metallic Ag-represented by the Ag^0 (Ag NP) standard - was detected in the Ag_2S exposed roots in Lufa soil but was absent in the corresponding roots in Woburn (Fig. 2). Previous studies have shown interconversion between Ag forms can occur in and on plant roots,[3] making it difficult to offer a simple interpretation for this lack of metallic Ag in roots grown in Woburn soil using our methodology, despite this Ag form being (somewhat) present in bulk soil (Fig. 2). The identification of Ag forms in tissues was limited by the standards chosen for LCF analysis, which were informed by current understanding of Ag speciation in soil and Ag uptake in plants. Therefore, other forms may be present but were not identified here. The levels of Ag_2S were not particularly surprising given the suite of chemical reactions that bind Ag to S in soil[46] and the potential for uptake of sulphidised forms given the utilisation of sulphur by plants for a range of biological processes.[47] The Ag-thiol complex present in all exposures could have been the result of a detoxification process in the root tissue. Ag has been shown to increase thiol levels in root extracts in the form of glutathione[37] and is a strong inducer of phytochelatins (an oligomer of glutathione).[48]

To complement XAS analysis, single particle-ICP-MS (spICP-MS) has been used in NP uptake studies in plants to provide an indication about the physical state of the NPs and to distinguish between nanoparticulate or dissolved metal forms.[49–51] It was applied here to quantify particulate Ag (>20–25 nm) in wheat tissues. XAS indicated that the Ag in roots was thiol or S bound (Fig. 2), suggesting that, despite a proportion of metallic Ag remaining in the bulk soils, the Ag sequestered within the root tissues was derived from dissolved or transformed particles. This is supported by the spICP-MS analysis indicating that the majority of the Ag found associated with the roots in both soils was not in particulate form (Fig. 3), suggesting dissolution of the Ag particles on the root surface prior to entry or during residence in tissues. This pattern was consistent across all soils and Ag forms, indicating that transformation to non-particulate Ag was independent of initial speciation. Comparing roots exposed to the different Ag forms, the roots exposed to Ag_2S NP had significantly greater ($p < 0.02$) particulate fractions of Ag than in the Ag NP and $AgNO_3$ exposures within each soil. This was more pronounced in Woburn soil, consistent with the slower transformation observed in the soil phase. Similar results have been reported in *Brassica napus* roots exposed to Ag NP (particulate fraction ~12%).[52]

As well as direct uptake, particulate Ag in roots could derive from the reduction of Ag^+ by humic acids[53] in the soil or rhizosphere, or within plant tissues following Ag^+ uptake and *in planta* reduction.[3,45,54–56] Some ionic Ag in roots may originate from Ag NP dissolution within plant cells, as toxicity associated with Ag NP exposure in some cases cannot fully be explained by NP dissolution in soil alone,[57] suggesting that Ag NP uptake followed by *in planta* dissolution also contributed to toxicity. A similar mechanism was proposed to partially explain toxicity following direct uptake of Ag_2S NP by exposed cucumber and wheat.[3,45] The slow release of Ag^+ from within plant cells would bind rapidly

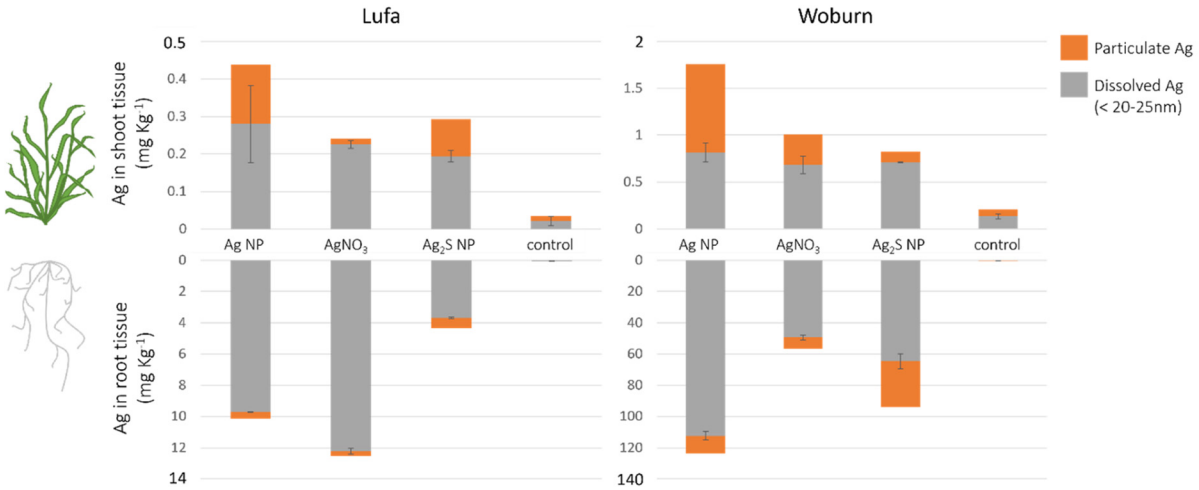

Fig. 3 Nanoparticulate (orange) and dissolved Ag (<20–25, grey) fractions of total Ag in root and shoot tissues of wheat exposed to Ag forms. Data presented as mean ± Std. dev for particulate Ag form. Note the difference in axis values for each soil.

to ligands, this could account for the Ag-thiol bound speciation found in the roots using the XAS analysis. Our study found that, despite large quantitative difference in Ag concentrations in the roots and shoots (Fig. 3), the proportion of Ag present in the shoots in particulate form was generally greater than that found in the roots. Particulate Ag was also seen in the shoots of cucumber and wheat exposed to $AgNO_3$ using spICP-MS, where *in planta* formed particles were smaller in size (35–85 nm) than the Ag_2S NP particles to which the plants were exposed.[45] Due to the similar NP size distributions in our experiment, it was not possible to distinguish between applied and *in planta* formed particles, and particles below the spICP-MS detection threshold would not have been quantified. However, the particulate Ag in $AgNO_3$ exposed plants is consistent with the formation of secondary particles either within the soil/rhizosphere or following uptake into plant tissues.

The XAS and spICP-MS data aligned well and detailed the nature of NPs and Ag speciation in both soils and plant tissues. The speciation data suggest that soil-driven differences in transformation dynamics, potentially related to Fe content and organic matter, may have contributed to differences in Ag bioavailability by promoting the persistence of particulate Ag and influencing interactions at the root-soil interface. Although slower transformation of Ag NPs in Woburn soil coincided with a greater particulate fraction in roots, this cannot be clearly attributed to enhanced uptake of intact nanoparticles and likely reflects a combination of interacting processes, including soil-particle interactions, Ag transformations, rhizosphere-mediated processes, microbial activity, and plant physiological responses, rather than a single dominant mechanism. Taken alone, these approaches do not provide a clear mechanistic explanation for the notably higher Ag uptake in wheat exposed in Woburn soil and further characterisation of soil-NP interactions and Ag speciation in the more bioavailable fractions (porewater) fractions may provide a better mechanistic understanding.

Conclusions

This study demonstrates that different forms of Ag are differentially bioavailable to wheat in soil exposures, and that Ag uptake is influenced by soil type. Although no single soil property was identified as the driver of the observed patterns, it is evident that both the form of Ag and soil properties must be considered when assessing the fate and effects of Ag NPs. Rather, the data support a combination of physicochemical properties interacting to regulate NP dissolution, sorption, transformation and retention within the rhizosphere. Previous studies have demonstrated Ag from Ag_2S NPs remain bioavailable to plants despite their comparatively low solubility and mobility.[3,33,44,45] This study demonstrates that the bioavailability of environmentally relevant Ag_2S NPs is strongly soil dependent and, in some soil contexts, may exceed that observed for more "mobile" forms of Ag, indicating that environmentally transformed nanoparticle forms cannot be assumed to exhibit uniformly reduced bioavailability across soils. This finding has clear implications for risk assessment, highlighting the importance of considering the test soils used to establish safe environmental levels for different Ag forms: the soil-driven variation in uptake observed here (*e.g.* Fig. 1) was greater than the variation between different Ag forms within any single soil. Therefore, bioaccumulation factors derived in one standard test soil are unlikely to be transferable across soils, and their use in prospective risk assessment would benefit from an explicit soil-variability term.

These results also show the importance of accounting for soil properties and Ag speciation in exposure modelling used for prospective assessments. For example, exposure models that adopt a dissolved or porewater concentration in the bulk soil as the bioavailable dose metric (by analogy with free-ion approaches developed for dissolved metals) may not capture Ag NP bioavailability if they do not account for nanoparticle delivery to and dissolution at the root-soil interface, rather

than relying on bulk solution speciation alone. Additionally, models that account for speciation typically treat sulphidation as an attenuation process yielding a comparatively immobile Ag pool. The bioavailability of Ag_2S NP demonstrated here indicates that such transformed species require their own bioavailability parameterisation rather than rely on their mobility as a proxy for bioavailability. Finally, because no single soil property explained the uptake patterns, the empirical property-based bioavailability normalisation established for dissolved metals is unlikely to transfer directly to NPs, requiring the development of bespoke NP bioavailability predictions.

Despite the divergence in Ag speciation between the different Ag forms in soils, we observed similar Ag speciation and particulate fractions in the roots, suggesting that the Ag uptake mechanisms in wheat are largely independent of initial Ag form. XAS analysis revealed that NPs in Woburn soil underwent slower transformation compared to those in Lufa soil, meaning NPs remain intact and likely more available for longer in Woburn soil. Further investigation of the mobile Ag fraction in the soils may help increase our understanding. While the exposure concentration used in this study enabled detailed characterisation of temporal Ag transformation, speciation, and plant uptake, it exceeds currently predicted environmental concentrations. Future studies at environmentally relevant exposure levels are needed to determine the extent to which Ag concentration influences concentration-dependent behaviours, such as homoaggregation and heteroaggregation, as well as the extent to which the soil-dependent patterns observed here are maintained. Collectively, these findings provide valuable insights into the behaviour of Ag NPs in natural soils, emphasising the need for soil-specific evaluations to advance environmental risk assessments for NPs.

Author contributions

A. G. E., E. L., D. S., M. M. and C. Sv. conceptualised the study. A. G. E., E. L., R. V., N. J. C., R. S., P. K., G. C. and M. G. P. developed the methodology. A. G. E., R. V., N. J. C., R. S., S. H., W. B., C. S., E. L., S. S., A. R., S. T., A. H., and H. G. performed the investigation. A. G. E., N. J. C., and R. S. conducted the formal analysis. A. G. E. wrote the original draft, and all authors contributed to writing, review and editing. C. Sv. acquired funding.

Conflicts of interest

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

Data availability

The datasets generated and/or analysed during this study are included in the Supplementary Information. These include detailed experimental methods, soil characterisation data, plant biomass measurements, nutrient and electrolyte concentrations in plant tissues, and total and particulate silver (Ag) concentrations in soils, plant tissues, and porewater. Additional data supporting the findings of this study, including X-ray absorption spectroscopy (XAS) datasets, are available from the corresponding author upon reasonable request.

Supplementary information is available. See DOI: https://doi.org/10.1039/d6en00494f.

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