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Storage and bioavailability of molybdenum in soils increased by organic matter complexation

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The micronutrient molybdenum is a necessary component of the nitrogen-fixing enzyme nitrogenase[1,2]. Molybdenum is very rare in soils, and is usually present in a highly soluble form, making it susceptible to leaching[3,4]. However, it is generally thought that molybdenum attaches to mineral surfaces in acidic soils; this would prevent its escape into the groundwater, but would also impede uptake by microbes[3]. Here we use X-ray spectroscopy to examine the chemical speciation of molybdenum in soil samples from forests in Arizona and New Jersey. We show that in the leaf litter layer, most of the molybdenum forms strong complexes with plant-derived tannins and tannin-like compounds; molybdenum binds to these organic ligands across a wide pH range. In deeper soils, molybdenum binds to both iron oxides and natural organic matter. We suggest that the molybdenum bound to organic matter can be captured by small complexing agents that are released by nitrogen-fixing bacteria; the molybdenum can then be incorporated into nitrogenase. We conclude that the binding of molybdenum to natural organic matter helps prevent leaching of molybdenum, and is thus a critical step in securing new nitrogen in terrestrial ecosystems.

The continued availability of essential trace elements to soil microbes, which catalyse key transformations in biogeochemical cycles, poses a perplexing conundrum: easily dissolved elements are rapidly leached away, whereas those that are tightly bound in minerals are not available for biological uptake. This problem is particularly acute for molybdenum (Mo), a cofactor in the nitrogenase enzyme responsible for nitrogen fixation. Mo is very rare in soils; it is also highly soluble in oxic systems. Here we show, using a combination of X-ray spectroscopy and measurements of metal bioavailability, that the pool of Mo bound to natural organic matter (NOM) is an important reservoir of Mo for N_2-fixing bacteria in soils. Over a wide pH range, Mo forms strong complexes with catechol groups in tannin-like compounds present in NOM, particularly in the leaf litter. Deeper in soils, Mo partitions between iron oxides and NOM. The NOM-bound Mo can be easily captured by small complexing agents released by free-living N_2-fixing bacteria and taken up for use in nitrogenase. By maintaining in surface soils a pool of Mo that is not readily leached but can be accessed by N_2-fixing bacteria, the binding of Mo to NOM probably has a critical role in the input of new nitrogen to terrestrial ecosystems.

Biological nitrogen fixation, which is carried out by specialized bacteria called diazotrophs, is the reaction that reduces atmospheric N_2 into ammonia[1], thereby providing new nitrogen to terrestrial ecosystems. N_2 fixation is carried out by symbiotic bacteria in higher plants, but a significant fraction is also contributed by free-living bacteria in soils. The reaction is catalysed by the enzyme nitrogenase[2], the most common and efficient form of which requires Mo as a cofactor in addition to iron (Fe). Mo, the main form of which in oxic systems is the highly soluble oxoanion molybdate (MoO_4^{2-}; ref. 3), is the least abundant of the biologically essential transition metals in soils[4]. Indeed, there is mounting evidence that Mo may be a limiting nutrient for N_2 fixation in pristine terrestrial ecosystems[5–7]. As molybdate has little affinity for most organic compounds[8], it is generally believed that MoO_4^{2-} in soils is adsorbed on mineral surfaces, particularly iron oxides[1]. Yet there is evidence that Mo in soils might also be associated with humic material[3,9,10] or polyphenolic compounds such as tannins[11,12], which are abundant in organic-rich soil horizons. The elucidation of Mo speciation is key to understanding what controls the availability and binding of Mo in soils, and hence, potentially, the rate of N_2 fixation: how Mo is bound to soil material determines how readily it can be leached by percolating water and whether it can be acquired by N_2-fixing bacteria.

We collected soil samples from a naturally Mo-rich area in the Coronado National Forest (Arizona) ([Mo] = 20–100 ppm, M. Chaffee, personal communication; Supplementary Table S1) to investigate Mo spatial distribution and chemical speciation by micro-X-ray fluorescence (μXRF) mapping. In view of the postulated importance of Mo adsorption on Fe and Mn oxides, the spatial distributions of Mo in the topsoil and in the subsoil were compared with those of Fe and Mn. μXRF mapping showed that certain soil areas with a high Mo content correlate well with Fe and/or Mn, whereas others do not (Fig. 1a). Many of the micro-X-ray absorption near-edge structure (μXANES) spectra of Mo collected from the topsoil and subsoil show features that are consistent with adsorption of Mo by haematite. Interestingly, this is true even in spots where the iron concentration is relatively low (Fig. 1b). A few of the spectra measured in the topsoil are different, however, indicating a different type of binding. The organic matter content of the topsoil is high, suggesting that organic matter might contribute to the binding of Mo in these samples. However, the relatively low Mo concentration of these soil samples, compared with the sensitivity of μXANES, precludes a more detailed analysis such as identification of binding groups.

We also collected an intact soil core from a temperate hardwood forest in New Jersey, separated the topsoil and subsoil, and spiked both with 0.2 mM molybdate. As observed in Arizona soils, the Mo XANES spectra of these samples clearly show that Mo adsorption to iron oxides, although important, is not the only process affecting Mo speciation (Fig. 2a). To investigate the importance of Mo binding to NOM, we extracted organic matter from the leaf litter layer collected in the Pine Barrens forest (New Jersey) and

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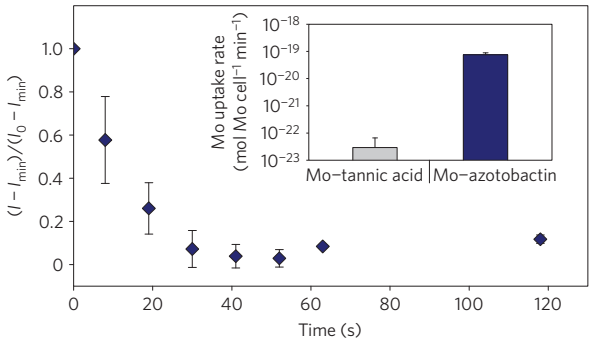

Figure 3 | Bioavailability of Mo bound to tannic acid. Decrease in fluorescence intensity over time of a solution of azotobactin to which a solution of Mo and tannic acid has been added at time = 0. The quenching of fluorescence of azotobactin corresponds to the formation of the Mo–azotobactin complex. Inset: Short-term uptake rates of Mo–tannic acid and Mo–azotobactin by Mo-limited *A. vinelandii* cells (mutant strain F196). The uptake rate of Mo–tannic acid is not significantly different from zero. The error bars represent the means ± minimum/maximum of two experiments.

promote its mobilization by producing organic acids[22] that enhance rock weathering. Mo, which in non-nodulating plants is used primarily in nitrate reductase for nitrate assimilation, is taken up by the trees through their dense root network, and part of it is deposited in leaves[23]. As the leaves fall to the ground and decompose, tannins and tannin-like compounds, which are an essential compound of highly productive soils, are released[17] and bind Mo. Mo binding to these tannin-like compounds occurs over a wide pH range and reduces leaching of the molybdate ion. This process, which keeps Mo concentrated in the top layers of soil, may be particularly important in circumneutral and alkaline soils, where there is little Mo adsorption on mineral surfaces[9,10,21]. In turn, nitrogen-fixing bacteria, many of which live in the O-horizon primarily composed of litter[24,25] and decomposing organic matter which are rich in polyphenols[26], excrete metallophores that outcompete tannins and bind Mo, making the metal available to the bacteria. Mo is then incorporated into the nitrogenase enzyme for nitrogen fixation. Ultimately, the fixed nitrogen penetrates the soil and promotes plant growth. In an ecosystem, the overall impact of higher plants on Mo cycling is thus potentially critical to the creation of a soil environment favourable to nitrogen fixation.

Methods

Collection and preparation of soil samples. Naturally Mo-rich soil samples collected in Arizona (USA) were analysed without further manipulation. In central New Jersey (USA), topsoil and subsoil (collected 50 cm below ground) samples were mixed with a 0.2 mM aqueous solution of Na_2MoO_4 (1 g soil in 10 ml water, pH = 5.7) and collected by filtration before analysis by X-ray spectroscopy.

Leaf litter layer samples collected in the Pine Barrens (New Jersey, USA) were ground before water extraction. The leaf litter extract was obtained as the filtrate of the resulting suspension and spiked with 1 mM molybdate. When needed, the pH of the leaf extract was adjusted with 0.1 N NaOH.

Preparation of standard Mo compounds. Mo adsorption on iron oxides was investigated by adding haematite or goethite to an aqueous solution of Na_2MoO_4 (pH = 4.5 ± 0.2). The solid was collected by centrifugation and rinsed with water before X-ray analysis.

The 1:1 Mo–malic acid complex was prepared in aqueous solution by adding 10 mM of Na_2MoO_4 to a 100 mM malic acid solution at pH = 4.7 (ref. 27). EXAFS analysis showed that the 1:1 Mo–malate complex is dominant under these conditions (data not shown).

The 1:1 Mo–azotochelin complex was prepared in aqueous solution by adding 1.0 mM Na_2MoO_4 to a 3.0 mM solution of azotochelin at pH = 6.1 (ref. 15).

Crystals of (di-μ-oxo-*bis*-{oxo[cysteinato(2-)]aquo-molybdate (V)} trihydrate) were prepared from aqueous solutions of molybdenum(VI) and cysteine[28].

The orange–red Mo–tannic acid complex was precipitated by titrating a solution of tannic acid in methanol with an aqueous solution of Na_2MoO_4 (ref. 12).

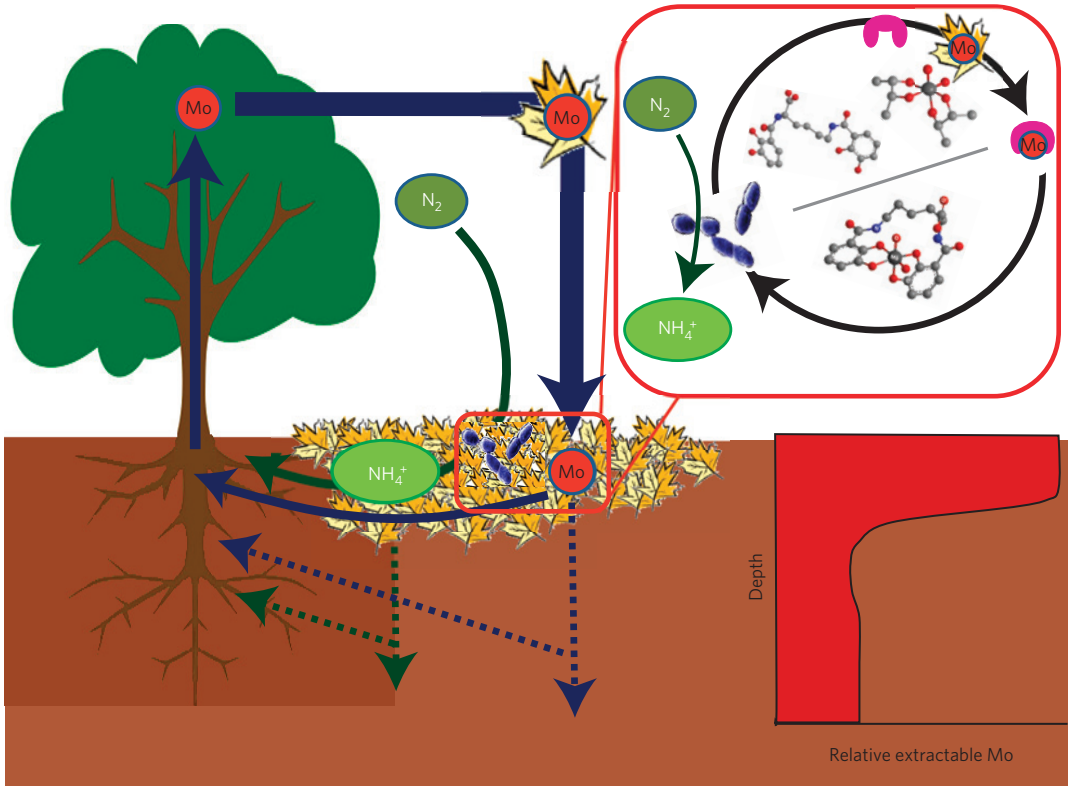

Figure 4 | Terrestrial cycle of Mo. The Mo present in the deep soil horizons is extracted by the root network of the trees, and is incorporated in the leaves. When the senescent leaves fall to the ground, they provide a Mo-enriched environment to N_2-fixing bacteria living in the upper soil horizon. Binding of Mo to leaf organic matter reduces Mo leaching rates from the soil, keeping Mo in the soil environment where it can be used by the bacteria. In turn, the new nitrogen fixed by the bacteria fertilizes tree growth, resulting in a classic mutualistic relationship. Bottom right: Idealized profile of Mo in soils[17].

X-ray analysis. XANES, EXAFS and μXRF measurements were carried out at beamlines X18B and X26B at the National Synchrotron Light Source (Brookhaven, New York, USA) and beamlines 11-2 and 4-3 at the Stanford Synchrotron Radiation Lightsource (see Supplementary Information for details).

Formation of Mo–azotobactin in the presence of tannic acid. A solution of molybdate and tannic acid was added to a solution of the fluorescent metallophore azotobactin[20] and the fluorescence was monitored over time (final concentrations: [Mo] = 9.7×10^{-6} M, [tannic acid] = 3.0×10^{-5} M, [azotobactin] = 1.4×10^{-6} M, pH = 6.6).

Bacterial uptake of Mo. *A. vinelandii* (mutant strain F196, which produces only the siderophore azotobactin[29]), was grown under diazotrophic conditions in Fe-replete and Mo-limited medium (Fe = 5×10^{-6} M and Mo = 2×10^{-8} M). Short-term uptake rates of Mo were measured as previously described in resuspension medium containing either [Mo] = 2×10^{-8} M and [tannic acid] = 3×10^{-6} M or [Mo] = 2×10^{-8} M and [azotobactin] = 10^{-6} M (pH = 6.6; refs 19, 30).

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Author contributions

All authors contributed extensively to the work presented in this letter.

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