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ORIGINAL ARTICLE

Growth response characteristics of alfalfa (*Medicago sativa* L.) grown in soil artificially contaminated with vanadium and soil naturally rich in vanadium

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Abstract

Plant growth responsive characteristics are critical to evaluate the metal resistance of the plant, especially for elements whose essentiality for higher plants have not yet been clearly defined until now. Therefore, an indoor pot experiment was conducted for alfalfa (*Medicago sativa* L.) grown in clean soil exogenously supplied with artificial source of soluble vanadium [0 (control), 75, 150, 300, 600, 900 mg kg^{-1}] and in the soil naturally rich in vanadium from a mining area (marked as M_0), respectively. Versus control, alfalfa growth was markedly influenced at ≥ 150 mg kg^{-1} exogenously supplied vanadium and M_0 treatment. The inhibited alfalfa growth at M_0 treatment may incorporate multifactor due to complicated components of the vanadium-rich soil from the mining area. Vanadium translocation capability of the alfalfa at M_0 treatment was significantly higher than that at the exogenous vanadium-addition treatments. The total uptake of vanadium in the alfalfa increased significantly at 75−300 mg kg^{-1} vanadium treatment, while no apparent difference arose at M_0 treatment versus control. The percentage of root vanadium uptake to the total amount markedly increased and later decreased marginally with vanadium concentration; a converse changing trend of the aboveground parts was noted. In addition, Proteobacteria were the most abundant bacteria community at all treatments excluding 900 mg kg^{-1} exogenous vanadium treatment. Actinobacteria, Chloroflexi, Gemmatimonadetes, and Acidobacteria were relatively abundant bacterial communities in soil with vanadium addition treatments. Alfalfa exhibited the potential to colonize in the vanadium-rich soil from natural/artificial sources by modulation of its vanadium bioaccumulation and translocation capability.

Keywords Alfalfa · Vanadium · Accumulation · Soil

Introduction

Vanadium is a known metal with high importance in physiology, environment, and industry (Kioseoglou et al. 2015). The vanadium concentration in the continental crust is averaged around 97 mg kg^{-1} (Schlesinger et al. 2017). Vanadium ions own many structural roles due to their structural and electronic analogousness with phosphorus (Crans et al. 2004). In addition, vanadium compounds may be used as an auxiliary therapy to the prescribed treatment of COVID-19 (Semiz 2022). Vanadium, at low levels, facilitates numerous biological processes, e.g., nitrogen fixation and halide oxidation; however, high dosages of vanadium are detrimental to exposed populations and counted as a contaminant (Sun et al. 2020). Ubiquitous anthropogenic sources, e.g., metalliferous industries, mining, vehicular exhaust, agricultural producing practices, coal/oil combustion, and atmospheric dry/wet deposition, constitute a nonnegligible input to soils for heavy metals (Liang et al. 2017). Similarly, anthropogenic activities, such as the mining and smelting for navajoite, extensive use of vanadium-bearing products, wastewater discharge from the mining, and fossil fuel combustion, have rendered substantial vanadium spread into the regional geological environments (Chen and Liu 2017; Wang et al.

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2020).Due to increasing human-derived enrichment globally, vanadium has become a contaminant of emerging attention (Chételat et al. 2021). Vanadium contamination has been experienced as a global environmental problem (Wang et al. 2022; Watt et al. 2018).

Environmental pollution of vanadium may induce an array of health problems involving humans, animals, and plants (Ali et al. 2020). Vanadium would engender the lesion in the human being's central nervous system, kidneys, and heart (Zhang et al. 2021). Ingestion of high concentrations of vanadium would be carcinogenic and toxic for humans and animals, even though it is essential when intaking in low quantities (Hudson-Edwards et al. 2019; Yang et al. 2014). However, the essentiality of vanadium for higher plant growth is still an open question (Imtiaz et al. 2015a; Wang and Liu 1999). In general, low quantities of vanadium were conducive to higher plant growth, such as promoting chlorophyll synthesis, nutrient intake, nitrogen assimilation, and potassium usage (Aihemaiti et al. 2020). Conversely, high dosages of vanadium adversely influence plant growth, including seed germination, subsequent young seedling growth, and final reproductive growth (Gokul et al. 2021; Imtiaz et al. 2018; Wu et al. 2021a, b; Yang et al. 2017b; Yuan et al. 2020).

Alfalfa (*Medicago sativa* L.), also named lucerne, is the most important forage legume globally due to its numerous superior traits like wide adaptability, high forage yield, desirable quality, and tolerance to frequent harvests (Bhattarai et al. 2021). In addition, alfalfa gained interest in remediation strategies for its rapid growth, high biomass yield, strong metal tolerance, high metal amassing in the root system, and capacity to form a symbiosis with rhizobacteria (Raklami et al. 2019, 2021). Growth-responsive characteristics of plants are critical parameters for evaluating their heavy metal tolerance. Moreover, the closer the growth environment of plants is to reality, the more accurate the growth response characteristics of plants will be, hence better serving the practice. Therefore, mimicking plant growth responses in contaminated soil from mining areas and even the trial in the contaminated site would be a step forward. Previous works have explored the growth response characteristics of alfalfa grown in soil artificially contaminated with vanadium (Gan et al. 2021; Yang et al. 2011). However, comparative research for alfalfa grown in soil from mining areas and artificially vanadium-contaminated soil is rare, which may provide more viable guidance for plant colonization and ecological restoration in actual vanadium-contaminated soil. Taking the above consideration into account, the aims of the present study are, therefore, to (1) assess the growth performance of alfalfa in artificially vanadium-contaminated soil and naturally vanadium-rich soil, (2) ascertain vanadium accumulation, translocation, and allocation characteristics within plants in artificially vanadium-contaminated soil and naturally vanadium-rich soil, and (3) explore the change in rhizospheric soil microbial community and vanadium fractions in the soils.

Materials and methods

Experiment setup

The examined contaminated and clean soil samples were collected respectively at the eastern district (Zhujiabaobao mining area) and Renhe district in Panzhihua city from Sichuan province of China. Panzhihua city (E101°15′ – 102°08′, N26°05′ – 27°12′) is at the confluence of Yalong River and Jinsha River (Teng et al. 2011). China's vanadium output is over 70,000 tons per year, and the primary vanadium resources are distributed in Sichuan, Anhui, Gansu, and Hunan provinces (Yang et al. 2017a). The Panzhihua region possesses 11% of vanadium reserves worldwide (Yang et al. 2014).

The basic properties of the contaminated and clean soil sampled are shown in Table 1. The collected soil samples were naturally air-dried and sieved to ≤ 2 mm (viz., 10 mesh). This study was performed in the form of an indoor pot experiment. The average room temperature was 28 °C during the day and 18ºC at night with a time duration of (14 ± 1) h and (10 ± 1) h in day/night. An aliquot of 1.5 kg of sieved sample was added to each polyethylene plastic container (16 cm of upper inner diameter, 13.5 cm in height). Exogenous vanadium concentration gradients were set based on our previous study (Liao and Yang 2020) and a pre-trial. The sampled clean soil with a vanadium background value of 96.28 mg kg^{-1} was used for control treatment with no exogenous vanadium addition. The clean soil of each pot was spiked exogenously with a stock solution of vanadium salt ($NaVO_3$) and consequently obtained a set of concentration gradients of spiked vanadium at 0 (control), 75, 150, 300, 600, and 900 mg kg^{-1}, respectively. Before vanadium addition, the required amount of vanadium ($NaVO_3{\cdot}2H_2O$) was calculated according to the concentration of exogenously spiked vanadium. Subsequently, the required vanadium solution was evenly sprayed in the soil of each pot. The vanadium-rich soil with a vanadium concentration of 385.56 mg kg^{-1} from the mining area without artificial vanadium spiking was taken as another vanadium concentration gradient treatment and designated M_0. Totally seven treatments were designed, and each treatment was replicated three times.

Before the experiment, the soil in each pot was watered with distilled water to keep 90% field water capacity, and then the pots were semi-sealed with preservative film and placed in a dark environment aging for 70 d. After soil aging, fifty healthy and uniformly sized alfalfa seeds were

Table 1 Basic properties of the sampled soil

Soil	pH	ω(Organic matter)/g kg^{-1}	CEC/cmol kg^{-1}	Total N/(g kg^{-1})	Total *P*/(g kg^{-1})	Total K/(g kg^{-1})	Exchangeable K/(mg kg^{-1})	Available *P* / (mg kg^{-1})	Hydrolyzable N/(mg kg^{-1})	ω Vanadium)/g kg^{-1}
Clean soil	6.75	4.29	17.39	0.34	0.53	16.26	13.63	14.2	8.58	96.28
Contaminated soil	6.94	5.85	30.87	0.21	0.22	2.67	3.52	0.93	7.2	385.56

selected and evenly sowed in each pot. The young plantlets of almost 2 cm in height after seed germination were thinned to 20 individuals per pot and used for subsequent treatment. During the experiment, the water loss of each pot was supplemented by weighing and watering to 70% of the field water capacity every 3 days. The position of each pot was randomly changed during watering to lower the errors caused by the local microenvironment of the experiment. All alfalfa seedlings were reaped after three months of treatment, and each reaped alfalfa individual was divided into three parts: root, stem, and leaf. The aboveground tissues (also named shoot) include two parts: leaf and stem. The same parts of the alfalfa seedlings of each pot were collected together, respectively.

Indicator determination

Vanadium concentration in the plant and soil

Fresh alfalfa tissues were dried (at 80 °C) to a constant weight in an oven when the experiment was terminated. The soil samples of each pot were air-dried. The dry alfalfa tissues and soil samples were digested separately in an intelligent microwave digester (TOPEX + , PREEKEM, China). The resulting digests were diluted with second deionized water. Vanadium concentration in the diluted digested solution was determined with an ICP-MS (NexION 300x, PerkinElmer, American). In the process of the determination of vanadium concentration in soil and plant samples, the quality control was performed by using soil (GBW07421) and plant (GBW10021) standard reference materials from the Institute of Geophysical and Geochemical Exploration (Beijing, China). The standard recovery range of vanadium concentration in the soil and plant samples was 94.5% to 105.3% and 93.1% to 104.8%, respectively.

Vanadium bioaccumulation and translocation factor

The vanadium translocation factor (TF) was calculated as the ratio of vanadium concentration in the alfalfa aerial part to that in the plant root (El-Meihy et al. 2019; Meng et al. 2022). The vanadium bioaccumulation factor (BF) was calculated as the ratio of vanadium concentration in alfalfa roots to that of the soil where the plants were grown (Rezapour et al. 2019; Xu et al. 2022).

Vanadium speciation in the soil

Vanadium speciation was analyzed according to the sequential extraction analysis method by Tessier et al. (1979).

Microbial community composition in the soil

The soils adhered to the alfalfa roots were collected after manually shaking them off the root surface. The collected soils were stored at 4 °C in a thermostatic refrigerator to analyze microorganism classification.

The genomic DNA of the rhizospheric soil samples was extracted via Mo Bio kit, a soil DNA extraction tool from MO BIO Laboratories (Carlsbad, Carbonic Anhydrase, USA). The DNA purity and concentration were detected by an agarose gel electrophoresis and a Nanodrop (NanoDrop 2000, Thermo Scientific, America). An appropriate amount of extracted DNA sample was put in a centrifuge tube, and the sample was diluted to 10 ng μL^{-1} with sterile water. The diluted genomic DNA was used as a template. The PCR was performed using specific primers (containing barcode) and the highly efficient high-fidelity enzyme (TaKaRa, Dalian) based on the selection of sequencing region to ensure amplification efficiency and accuracy. Agarose (mass fraction 1%) gel electrophoresis was used for PCR product detection. The target band was recovered by a DNA gel extraction kit provided by Qingke Company, and the Nanodrop was used for the DNA concentration and quality determination. Library construction was performed with TruSeq® DNA PCR-free sample preparation kit, and simultaneously Qubit and qPCR were used to quantify the constructed libraries. The sequence underwent on the Illumina sequencing platform after the constructed libraries were qualified.

Statistical analysis

The data analysis was performed with Microsoft Excel 2013. Analysis of variance underwent one-way ANOVA by Statistical Analysis System (SAS) version 9. The means were compared among different vanadium concentration treatments at the $P < 0.05$ significant level.

Results and discussion

Effect of vanadium on alfalfa plant height and root length

Treatments of exogenous vanadium application of ≥ 150 mg kg^{-1} significantly influenced alfalfa growth (Fig. 1). The alfalfa plant could not survive at 900 mg kg^{-1} of vanadium treatment. No dead plant was found when the exogenous vanadium application was ≤ 600 mg kg^{-1}. However, alfalfa growth was severely inhibited when the exogenously added vanadium concentration was 600 mg kg^{-1}. Growth changes in plant height and root length at all treatments are illustrated in Fig. 2. No striking difference occurred in plant height and root length at 75 mg kg^{-1} vanadium-spiked treatment versus control. Simultaneously, the plant root length at M_0 treatment was not strikingly inhibited relative to the control. Alfalfa seedling height at 150, 300, and 600 mg kg^{-1} vanadium addition and M_0 treatment was conspicuously decreased by 61.16%, 73.46%, 88.58%, and 70.65% compared with the control,

Fig. 1 Growth performance of alfalfa after the termination of the experiment. 'Control' indicates alfalfa grown in clean soil sampled without exogenously added vanadium. '75, 150, 300, 600, and 900 mg kg^{-1}' represent the clean soil sample exogenously spiked with 75, 150, 300, 600, and 900 mg kg^{-1} vanadium. 'M_0' indicates the treatment alfalfa grown in vanadium-rich soil taken from the mining area

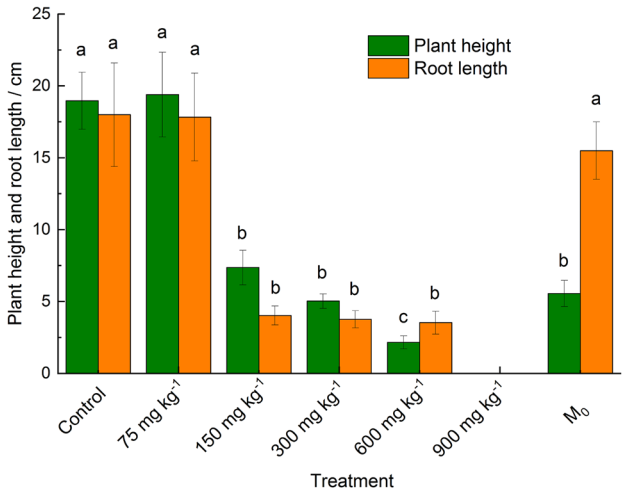

Fig. 2 Effect conferred by vanadium on alfalfa seedling height and root length. Different lowercase letters on the histograms represent the significant ($P < 0.05$) differences in alfalfa seedling height or root length among different treatments. The vertical error bar in the columns signifies standard deviation ($n = 3$). 'Control' indicates alfalfa grown in clean soil sampled without exogenously added vanadium. '75, 150, 300, 600, and 900 mg kg^{-1}' represent the treatments for clean soil samples exogenously spiked with 75, 150, 300, 600, and 900 mg kg^{-1} vanadium. 'M_0' indicates the treatment of alfalfa grown in vanadium-rich soil from the mining area. No data was recorded at 900 mg kg^{-1} vanadium treatment because of the very low biomass or the death of alfalfa

respectively. Besides, the root length noticeably declined by 77.59%, 79.07%, and 80.37% relative to control at 150, 300, and 600 mg kg^{-1} vanadium treatments, respectively.

Effects of vanadium on plant height and/or root length have been reported previously (Garau et al. 2015; Imtiaz et al. 2015b; Nawaz et al. 2018; Saco et al. 2013; Yang and Tang 2015). The general conclusion can be drawn that low dosages of vanadium is conducive to plant height (García-Jiménez et al. 2018) and root length (Altaf et al. 2022); nevertheless, higher concentrations of vanadium is detrimental to plant growth involving plant height and root length (Imtiaz et al. 2015b). In addition, Rosso et al. (2005) noted that vanadium remarkably suppressed the plant height and stem thickness of *Salicornia virginica*. The effects resulting from vanadium on plant height were correlative with the vanadium exposure time (García-Jiménez et al. 2018). Additionally, vanadium decreased the secondary and tertiary lateral branching and increased the thickness of the primary root of cuphea (Olness et al. 2005).

Vanadium accumulation, translocation, and distribution in alfalfa

Vanadium concentration in alfalfa root, stem, and leaf ascended with increasing vanadium dosage spiked exogenously (Fig. 3). Root vanadium concentrations at 0, 75, 150, 300, 600 mg kg^{-1} vanadium, and M_0 treatment were 4.38, 40.42, 204.58, 809.69, 1266.69, and 16.19 mg kg^{-1}, respectively, which were 1.83, 5.86, 13.16, 5.09, 3.19, and 1.63 times of the corresponding vanadium concentration in stem (2.40, 6.90, 15.54, 159.17, 396.49 and 9.92 mg kg^{-1}), and were 5.76, 23.23, 91.74, 43.46, 28.76, and 6.45 times of the corresponding vanadium concentration in leaf (0.76, 1.74, 2.23, 18.63, 44.05 and 2.51 mg kg^{-1}). There was no noticeable difference in vanadium concentration in the same tissues (root, stem, and leaf) of alfalfa among control, 75 mg kg^{-1} vanadium spiking, and M_0 treatment. Vanadium concentration in various parts of alfalfa was sequenced as root > stem > leaf, and a similar plant vanadium accumulation order was also reported previously (Nawaz et al. 2018; Ray et al. 2020). IN addition, the tissue vanadium concentration of the pepper was ordered as leaf > root > stem when treated with 5, 10 µM of vanadium, and the order was root > leaf > stem when treated with 15 µM of vanadium in the hydroponic system (García-Jiménez et al. 2018). Hou et al. (2013, 2014) and Qian et al. (2014) also reported the order of vanadium accumulation of root > leaf > stem. Besides, the order of leaf > stem > root also occurred in a control treatment (García-Jiménez et al. 2018). In the present study, vanadium is mainly concentrated in alfalfa roots, and only trace amounts are transferred to the aerial tissues. Similar results were reported in Chinese cabbage (*Brassica rapa* L.) (Tian et al. 2014), chickpea (Imtiaz et al. 2016),

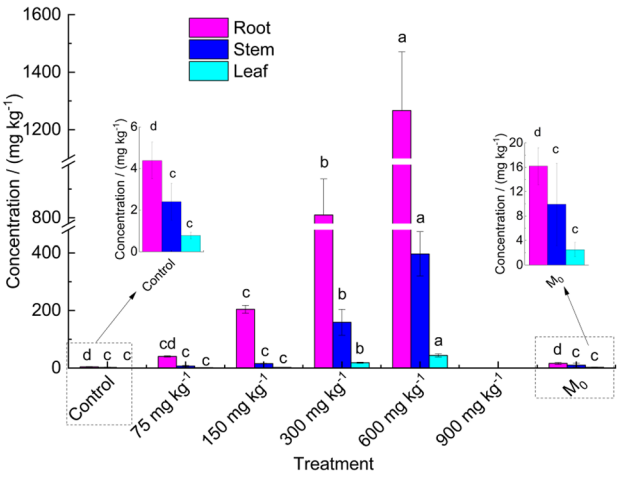

Fig. 3 Effects of vanadium on leaf, stem, and root vanadium concentration of alfalfa. Different lowercase letters on the histograms represent the significant ($P<0.05$) differences in vanadium concentration at varied treatments. The vertical error bar in the columns signifies standard deviation ($n=3$). 'Control' indicates alfalfa grown in clean soil sampled without exogenously added vanadium. '75, 150, 300, 600, and 900 mg kg^{-1}' represent the treatments for clean soil samples exogenously spiked with 75, 150, 300, 600, and 900 mg kg^{-1} vanadium. 'M_0' indicates the treatment of alfalfa grown in vanadium-rich soil from the mining area. No data was recorded at 900 mg kg^{-1} vanadium treatment because of the very low biomass or the death of alfalfa

maize (*Zea mays* L.) (Hou et al. 2019), and soybean (Yang et al. 2017b). Research by Hou et al. (2020) pictured that the cell wall provided the leading vanadium absorption site in corn roots and leaves. The cell wall plays a vital role in immobilizing toxic metal ions by giving pectic sites, histidyl groups, and cellular carbohydrates (like callose and mucilage), thus hindering the uptake of heavy metals into the cytosol (Manara 2012). Yuan et al. (2022) showed that the rice root cell wall stored 69.85−82.71% of vanadium that entered the root. Metals' storage in epidermal tissues may lessen the influence in more metabolically active tissues like mesophyll (Sheoran et al. 2011).

The TF of vanadium for alfalfa first decreased and then increased with increasing vanadium addition (Fig. 4). Conversely, the BF was first increased and then decreased with increasing vanadium addition level. The minimal TF (0.04) and the maximal BF (2.1) were respectively obtained at 150 mg kg^{-1} and 300 mg kg^{-1} vanadium addition treatments. Compared with the control, the TF significantly ($P<0.05$) decreased by 69.96%, 88.50%, 74.80%, and 61.85% at 75, 150, 300, and 600 mg kg^{-1} vanadium addition treatments, respectively. Significantly ascended BF of 15.90, 39.83, and 34.90 times occurred at 150, 300, and 600 mg kg^{-1} vanadium treatment relative to control. For M_0 treatment, no evident difference in BF and TF existed compared with the control. It was noteworthy that significantly high vanadium

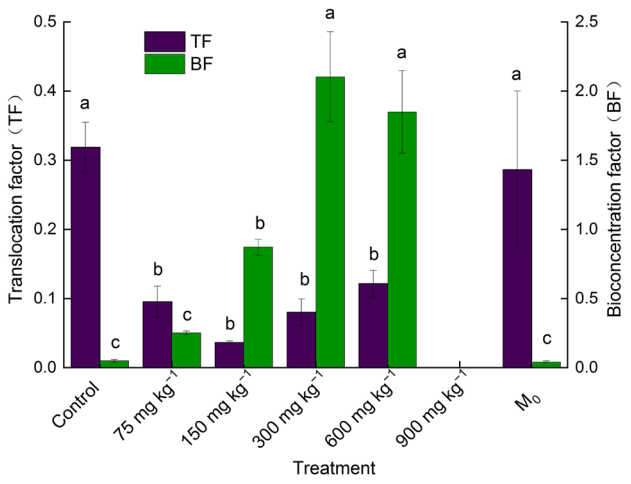

Fig. 4 Effects of vanadium on translocation factor (TF) and bioaccumulation factor (BF) of alfalfa. Different lowercase letters on the histograms represent the significant ($P<0.05$) differences in TF or BF among different treatments. The vertical error bar in the columns signifies standard deviation ($n=3$). 'Control' indicates alfalfa grown in clean soil sampled without exogenously added vanadium. '75, 150, 300, 600, and 900 mg kg^{-1}' represent the treatments for clean soil samples exogenously spiked with 75, 150, 300, 600, and 900 mg kg^{-1} vanadium. 'M_0' indicates the treatment of alfalfa grown in vanadium-rich soil from the mining area. No data was recorded at 900 mg kg^{-1} vanadium treatment because of the very low biomass or the death of alfalfa

translocation capability for alfalfa at M_0 treatment occurred versus exogenous vanadium (75, 150, 300, and 600 mg kg^{-1}) spiking treatments. *Setaria viridis* accumulated exceeding 1000 mg kg^{-1} vanadium in its aerial parts and simultaneously exhibited TF > 1 for vanadium (Aihemaiti et al. 2017). *Pteris vittata*, a vanadium accumulator, exhibited a low vanadium TF of 0.10 and 0.11 when grown in mining and smelting areas (Wang et al. 2018). In addition, *Zea mays* showed a BF > 1 for vanadium (Ameh et al. 2019). In brief, alfalfa exhibited relatively strong vanadium bioaccumulation (BF) and poor translocation (TF) capability when grown in vanadium-contaminated soil from the artificial source; by contrast, alfalfa showed a low BF though a high TF when grown in soil naturally rich in vanadium.

Effect of vanadium on dry matter of alfalfa

Overall, the dry matter mass yield in various tissues of alfalfa descended with the ascending exogenously added vanadium concentration (Table 2). Compared with the control, the dry matter mass in all tissues (except a slight increase in root dry matter) reduced insignificantly at 75 mg kg^{-1} vanadium-added treatment; by contrast, a striking reduction occurred when the spiked vanadium concentrations were ≥ 150 mg kg^{-1}. A previous study showed that low concentrations of vanadium increased while higher levels of vanadium decreased pepper root dry matter (García-Jiménez et al. 2018). Compared with the control, alfalfa biomass significantly ($P<0.05$) decreased by 72.07%, 82.07%, 91.86%, and 73.36% at 150, 300, and 600 mg kg^{-1} vanadium addition and M_0 treatment. Plant biomass is a good indicator of the overall health of the plant growing in the presence of heavy metals (Israr et al. 2011; Imtiaz et al. 2015b). The biomass reduction of plants resulting from vanadium was reported in some studies (Imtiaz et al. 2015b; Imtiaz et al. 2018; Wang and Liu 1999; Wu et al. 2022b). Moreover, in plant cellular systems, solutes and metabolites transport (into and out of the cell) is actuated predominantly by an H^+ electrochemical gradient produced by the plasma membrane (PM) H^+-ATPases (Wang et al. 2014). In other words, the PM H^+-ATPase is a powerhouse supporting plant growth and development (Mishra et al. 2022). Vanadate may depress the driving force of plants used for transporting nutrients and metabolites within the plant due to its inhibition of plasma membrane H^+-ATPase (Imtiaz et al. 2015a; Villegas et al. 2000; Wu et al. 2021b). Additionally, vanadium could inhibit plant water and mineral element uptake, which may

Table 2 Effect of different treatments on dry matter of alfalfa tissues

Treatments	Dry matter mass/g				
	Root	Stem	Leaf	Shoot	Whole plant
Control	0.255 ± 0.056^{a}	0.457 ± 0.052^{a}	0.677 ± 0.048^{a}	1.134 ± 0.099^{a}	1.389 ± 0.154^{a}
75 mg kg^{-1}	0.272 ± 0.060^{a}	0.455 ± 0.017^{a}	0.632 ± 0.086^{a}	1.087 ± 0.077^{a}	1.359 ± 0.018^{a}
150 mg kg^{-1}	0.103 ± 0.019^{b}	0.114 ± 0.021^{b}	0.172 ± 0.012^{b}	0.285 ± 0.033^{b}	0.388 ± 0.047^{b}
300 mg kg^{-1}	0.074 ± 0.007^{b}	0.057 ± 0.007^{c}	0.118 ± 0.012^{bc}	0.175 ± 0.019^{c}	0.249 ± 0.020^{c}
600 mg kg^{-1}	0.043 ± 0.004^{b}	0.022 ± 0.003^{c}	0.048 ± 0.002^{c}	0.070 ± 0.001^{d}	0.113 ± 0.004^{d}
900 mg kg^{-1}	–	–	–	–	–
M_0	0.200 ± 0.049^{a}	0.055 ± 0.009^{c}	0.116 ± 0.009^{bc}	0.171 ± 0.015^{c}	0.370 ± 0.063^{bc}

'–' represents that most of the plants died before the end of the experiment, and therefore no data was recorded. M_0 represents the treatment that alfalfa grown in contaminated soil (with no exogenous vanadium addition) sampled from the mining area. Means followed by the different superscript lowercase letters in the same column signify the significant difference for dry matter in the same tissues at $P<0.05$

be responsible for the decreased plant biomass yield (Furukawa et al. 2001; Kaplan et al. 1990). High concentrations of vanadium markedly lower the photosynthetic pigments (chlorophyll a, b, and carotenoids) and photosynthesis (Altaf et al. 2020, 2022), which also account for the reduced synthesis of dry matter mass of plants.

Vanadium uptake amount and the percentages of tissues uptake to total intake

Vanadium uptake amounts in alfalfa roots and whole plants ascended remarkably and later decreased slightly ($P > 0.05$) with exogenously spiked vanadium concentration. While the leaf, stem, and shoot vanadium absorption amounts fluctuated with increasing exogenously spiked vanadium concentration (Fig. 5). The maximized vanadium uptake amount of root (59.19 μg), stem (8.89 μg), leaf (2.18 μg), shoot (11.07 μg), and the whole plant (70.26 μg) was achieved at 300 mg kg^{-1} vanadium-added treatment. At M_0 treatment, vanadium intake amount in the root (3.18 μg), stem (0.52 μg), shoot (0.80 μg), and the whole plant (3.98 μg) was similar to the corresponding tissue intake quantity of the control plants. Total vanadium uptake amount of alfalfa at 0, 75, 150, 300, 600 mg kg^{-1} vanadium addition and M_0 treatment was 2.67, 15.13, 23.11, 70.26, 64.74, and 3.98 μg, respectively. At 0, 75, 150, 300, 600 mg kg^{-1} additive vanadium treatments and M_0 treatment, the root vanadium uptake amounts were 1.09, 10.93, 20.98, 59.19, 54.11, and 3.18 μg, respectively.

The percentages of root vanadium uptake amount to that of total intake quantity firstly increased significantly ($P < 0.05$) and then declined slightly ($P > 0.05$) with increasing exogenous vanadium addition (Fig. 6). Contrarily, the percentages of the stem, leaf, and shoot vanadium uptake amount to total intake quantity were firstly descended evidently ($P < 0.05$) and then elevated slightly ($P > 0.05$) with increasing exogenous vanadium spiking (Fig. 6). The maximal vanadium absorption percentage of root to total uptake amount, and correspondingly the minimal percentage of other portions (stem, leaf, and shoot), occurred at 150 mg kg^{-1} vanadium-added treatment (Fig. 6). The vanadium uptake percentages of the root to the total uptake amount were 41.23%, 72.00%, 90.68%, 84.25%, 83.53%, and 80.04% at control, 75, 150, 300, 600 mg kg^{-1} vanadium, and M_0 treatment, respectively. The percentages of vanadium absorption in roots to total intake amount at M_0 and vanadium-spiked treatments were remarkably ($P < 0.05$) higher than those of the control treatment (Fig. 6). At 75, 150, 300, and 600 mg kg^{-1} additive vanadium and M_0 treatment, the root vanadium uptake amount to total intake quantity significantly increased by 74.63%, 119.94%, 104.34%, 102.60%, and 94.13%, respectively, compared with the control. The high vanadium uptake percentage of the root to

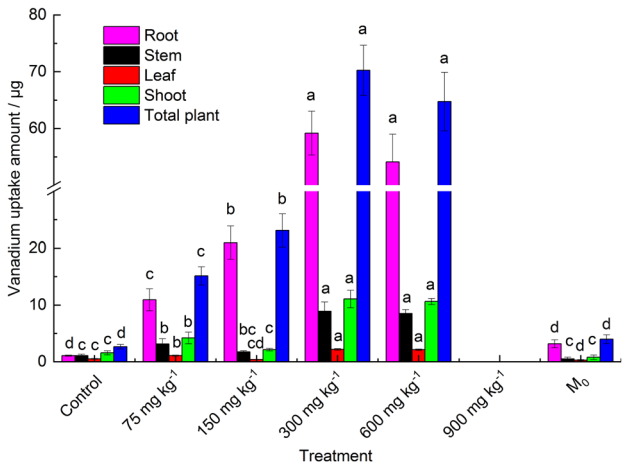

Fig. 5 Vanadium uptake amount in alfalfa tissues. Different lowercase letters on the histograms represent the significant ($P < 0.05$) differences in vanadium uptake amount at varied treatments. The vertical error bar in the columns signifies standard deviation ($n = 3$). 'Control' indicates alfalfa grown in clean soil sampled without exogenously added vanadium. '75, 150, 300, 600, and 900 mg kg^{-1}' represent the treatments for clean soil samples exogenously spiked with 75, 150, 300, 600, and 900 mg kg^{-1} vanadium. 'M_0' indicates the treatment of alfalfa grown in vanadium-rich soil from the mining area. No data was recorded at 900 mg kg^{-1} vanadium treatment because of the very low biomass or the death of alfalfa

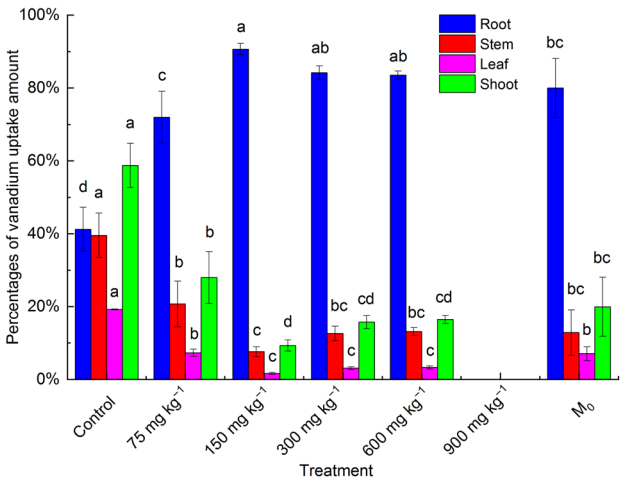

Fig. 6 The percentages of vanadium uptake amount in alfalfa tissues. Different lowercase letters on the histograms represent the significant ($P < 0.05$) differences in vanadium uptake amount percentages at varied treatments. The vertical error bar in the columns signifies standard deviation ($n = 3$). 'Control' indicates alfalfa grown in clean soil sampled without exogenously added vanadium. '75, 150, 300, 600, and 900 mg kg^{-1}' represent the treatments for clean soil samples exogenously spiked with 75, 150, 300, 600, and 900 mg kg^{-1} vanadium. 'M_0' indicates the treatment of alfalfa grown in vanadium-rich soil from the mining area. No data was recorded at 900 mg kg^{-1} vanadium treatment because of the very low biomass or the death of alfalfa

total alfalfa intake manifests the mechanism of vanadium uptake and storage by the root to protect aerial parts growth, especially when the vanadium concentration in the soil elevates ($\geq$75 mg kg^{-1}). To sum up, the major accumulation of vanadium by alfalfa root is not only reflected in root vanadium concentration (Fig. 3) but also in root vanadium uptake amount (Fig. 5) and uptake amount percentages (Fig. 6).

Vanadium speciation in the soil after growing alfalfa

The different vanadium speciation in the soil after growing alfalfa is shown in Fig. 7. The percentage of vanadium in the residual phase decreased, and other phases increased with increasing additive vanadium concentration. For the vanadium-rich soil of the mining area, the proportion of the residual vanadium was very high (> 85%), and the proportions of other fractions were pretty low. Most vanadium in the soil occurred in the residual fraction. A previous study also revealed that the residual fraction of vanadium accounted for 66.5% of total vanadium in lacustrine soil and 77.5% in fluvial soil in the north of Nile Delta (Egypt) (Shaheen et al. 2014). The vanadium percentage of the residual fraction occupied 93% of total vanadium in some soils (Huang et al. 2015). As shown in Fig. 3, vanadium levels in alfalfa tissues (root, stem, and leaf) were relatively low when the plants were grown in the vanadium-rich soil sampled from the mining area despite a relatively high vanadium concentration value (385.6 mg kg^{-1}) in the area. The possible reason for the scenario was the absolute dominance of vanadium in the residual fraction in the soil, which decreases the bioavailable vanadium for the plant. Assuredly, the absolute predominance of vanadium in the residual phase in the soil was conducive to alleviating plants' toxicity when the plant grew in the soil contaminated with vanadium. Generally, geogenic vanadium exhibits a lower solubility than exogenously spiked vanadium (Baken et al. 2012; Larsson et al. 2013). Ageing reactions in soils, viz., the long-term changing in solubility that occurs following prolonged periods, have been noted in many trace metals (Baken et al. 2012). Such aging reactions probably lower the mobility and bioavailability of chemicals (Baken et al. 2012). In addition, to a certain extent, the relatively poor alfalfa growth may be closely related to the low nutrient in the soils in mining areas (Table 1), which render the plants hard to biosynthesize more matter.

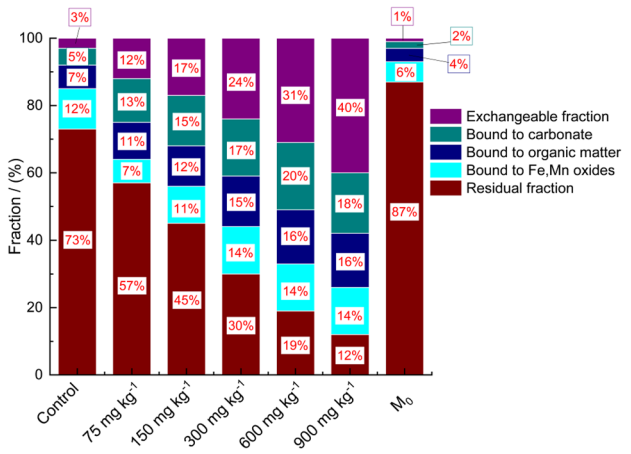

Fig. 7 Vanadium speciation grading changes in the soil after growing alflafa plants. 'Control' indicates alfalfa grown in clean soil sampled without exogenously added vanadium. '75, 150, 300, 600, and 900 mg kg^{-1}' represent the treatments for clean soil samples exogenously spiked with 75, 150, 300, 600, and 900 mg kg^{-1} vanadium. 'M_0' indicates the treatment of alfalfa grown in vanadium-rich soil from the mining area

Microbial community composition in the rhizosphere soil

Bacteria, the most abundant microorganisms in the earth, can dwell in various environmental conditions (Yin et al. 2019). Bacterial community composition according to phylum level in the rhizospheric soil after growing alfalfa is shown in Fig. 8. The rhizospheric soil microbial communities were mainly composed of Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, Acidobacteria, Firmicutes, Thaumarchaeota, Planctomycetes, Bacteroidetes, and Nitrospirae, from which Proteobacteria occupied the maximal proportion (excluding 900 mg kg^{-1} vanadium treatment) (Fig. 8). Macías-Pérez et al. (2022) reported approximate bacterial phyla classification in the bauxite residue samples.

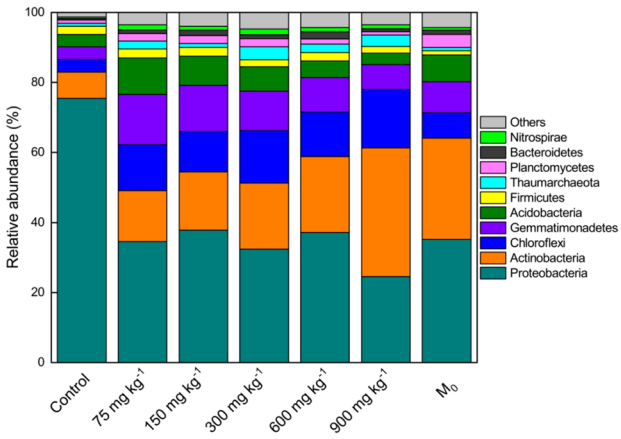

Fig. 8 Numerically dominant clades of microorganism community composition of rhizosphere soil after growing alfalfa plants (phylum level). 'Control' indicates alfalfa grown in clean soil sampled without exogenously added vanadium. '75, 150, 300, 600, and 900 mg kg^{-1}' represent the treatments for clean soil samples exogenously spiked with 75, 150, 300, 600, and 900 mg kg^{-1} vanadium. 'M_0' indicates the treatment of alfalfa grown in vanadium-rich soil from the mining area. Notes: 'Others' indicates the total abundance of other phyla that can not be confirmed at the phyla level

At 0 (control), 75, 150, 300, 600, 900 mg kg^{-1} additive vanadium, and M_0 treatment, the relative abundance of Proteobacteria, Actinobacteria, Chloroflexi, Gemmatimonadetes, and Acidobacteria were 24.51−75.42%, 7.57−36.85%, 3.60−16.63%, 3.62−14.45%, and 3.26−10.36%, respectively. Proteobacteria were the most abundant bacteria community at the control treatment, while the component percentages of other bacteria were pretty low. Proteobacteria are one of the largest phyla and simultaneously the most versatile in the bacteria domain (Nyoyoko 2022). Wu et al. (2022a) found that Proteobacteria were the dominant phyla with maximal relative abundance in the Southwest China mining tailings. Some Proteobacteria were considered to own multiple genes encoding heavy metal oxidase and participated in resisting heavy metals (Wu et al. 2022a). Recent research by Gan et al. (2022) also showed that Proteobacteria, Actinobacteria, and Chloroflexi were the dominant phyla in the soil and tailing samples of two vertical profiles in Majiatian tailing reservoir located in Panzhihua city, China. Actinobacteria may produce a range of secondary metabolites and degrade organic matter to improve the growth of plants in harsh habitats (Pan et al. 2021). Chloroflexi are cosmopolitan and abundant that live in free-living microbial communities, and one reason responsible for this is their metabolic diversity (Islam et al. 2019). Proteobacteria, Actinobacteria, and Acidobacteria own the capability of metallic ion transformation and(or) resistance, thereby allowing them to adapt to a vanadium-contaminated environment (Wang et al. 2021). The relatively high abundance of Gemmatimonadetes may be due to their capability to reduce V(V) to V(IV) (Fei et al. 2022). For Chloroflexi, it can utilize V(V) as an electron acceptor (Wang et al. 2020; Zhang et al. 2015).

Plant-related microbiome experiences dynamic adjustment in the composition and functional activities in fluctuating environments, which provides an intrinsic guarantee for the survival and health of plants (Saijo and Loo 2020). Rhizospheric microorganisms may promote plant growth, elevate metal availability and mobility in contaminated soils, and improve plant metal tolerance and accumulation content (Hou et al. 2017). The adsorbed heavy metal ions can be transferred to living bacterial cells in a metabolism-dependent way, thereby changing the redox state of heavy metal ions to reduce their toxicity (Yin et al. 2019). Many bacteria can use vanadium for varying biological functions (Rehder 2015). Research by Sun et al. (2018) showed that the rhizospheric microbial communities were strongly related to vanadium and chromium concentrations in multiple metal(loid)s contaminated soil. In addition, vanadium tolerance seems pervasive among bacteria, and this resistance was correlated with efflux pump and TCA cycle-related genes (Yelton et al. 2013). Since the V(V) is the most toxic speciation, some vanadium-tolerant (reducing) bacteria will become the dominant bacteria and participate in the plant detoxification mechanism in vanadium-contaminated soil. Reduction of vanadium from pentavalent vanadium [V(V)] to tetravalent vanadium [V(IV)] or trivalent vanadium [V(III)] can bring about the precipitation of vanadium-bearing minerals and thus lower the vanadium bioavailability and toxicity (Yelton et al. 2013). Microbe could reduce and immobilize V(V) (Hao et al. 2021; Li et al. 2022). Some bacteria can effectively reduce V(V) to V(IV), e.g., *Lactococcus raffinolactis* (Zhang et al. 2021). Zhou et al. (2022) found that extracellular reduction through extracellular polymeric substances (EPS) was the major V(V) removal process for *bacillus* sp. PFYN01, while the intracellular reduction underwent mediated by some intracellular reductases. Some functional genes (like omcA, omcB, and mtrC) and compounds (like cytochrome c, NADH) supported the V(V) reduction of bacteria (Shi et al. 2020a, b). Many microorganisms can resist high concentrations of vanadium, e.g., *Pseudomonas* and *Thiobacilli* could tolerate 5000 mg L^{-1} V(V) (Huang et al. 2015). Some vanadium-tolerant bacteria accumulate vanadium, possibly in the form of precipitates (Yelton et al. 2013). However, overdoses of vanadium in the soil can constrain plant and microorganism growth (Larsson et al. 2013; Zhang et al. 2018). Microbes are sensitive to heavy metal pollution, and the heavy metals pollution may provoke drastic alteration in microbial community composition and activity, leading to a decline in microbial diversity and the assembling of tolerant species through environmental filtering (Wang et al. 2020).

Conclusion

Compared with the control, alfalfa growth was markedly affected at ≥ 150 mg kg^{-1} vanadium-addition treatments and the vanadium-rich soil with 385.56 mg kg^{-1} vanadium from a mining area. The alfalfa plant could barely grow at 900 mg kg^{-1} vanadium-spiked treatment. Artificially vanadium-spiked soil and the vanadium-rich soil in the mining area reduced the relative abundance of Proteobacteria. The maximal vanadium absorption amount of the total plant occurred at 300 mg kg^{-1} exogenous vanadium-added treatment. Vanadium was primarily stored in alfalfa root and thus alleviated its toxicity on aerial portion growth. Briefly, alfalfa showed a relatively strong vanadium accumulation (BF) and poor translocation (TF) capability in artificially contaminated soil. Conversely, the plant embodied a relatively high TF but low BF in naturally vanadium-rich soil, which makes alfalfa a colonizer in vanadium-contaminated soil with the potential for eco-establishment. Altogether, alfalfa has the potential to remediate vanadium-loaded soil, even though the definite answer of whether it is feasible for the practice cannot be obtained directly for a vacancy of

the practical application information. Assuredly, obstacles impeding the implementation of fieldwork remediation trials should be overcome to test the remedial ability of alfalfa in reality in coming studies, and ultimately putting the alfalfa in vegetation restoration or eco-remediation practice of vanadium-contaminated soil. Of note, some ancillary measures may also need to be synchronized, e.g., site management and proper treatment for reaped remedial plants.

Author contribution statement ZW: data collect, methodology, formal analysis, validation, writing-original draft, writing—review and editing. YR: validation, writing—review and editing. AA: validation, writing—review and editing. JY: conceptualization, funding acquisition, supervision, validation, writing—review and editing.

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Data availability All data related to the present work can be obtained through Email: yanyang@scu.edu.cn.

Declarations

Conflict of interest The authors declare having no conflict of interest, including any financial, personal or other relationships with other people or organizations.

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