The differential physiological mechanism and ecological response of Pisolithus sp. mycorrhizal Pinus thunbergii to Chromium

Liang Shi
Binhao Liu
Xinzhe Zhang
Xuan Zhao
Zhenguo Shen
Yahua Chen (yahuachen@njau.edu.cn)
https://orcid.org/0000-0002-4028-1607

Research Article

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Abstract

Aims Pisolithus sp. as a class of ectomycorrhizal (ECM) fungi with multiple HM tolerance. However, the physiological mechanism and ecological response of *Pisolithus* sp. ECM plants to chromium (Cr) remain unclear. *Pisolithus* sp.1 (Cr tolerant strain) and *Pisolithus* sp.2 (Cr sensitive strain) ECM *Pinus thunbergii* were synthesized to explore their different physiological and ecological response to Cr in this work.

Methods Biomass, nutrient elements and Cr concentration of plants; Cr form and content in soil; Physicochemical properties, enzyme activities and microbial high-throughput analysis of soil were performed by pot and acute exposure experiments.

Results The growth and Cr tolerance of *P. thunbergii* were improved after been inoculated with *Pisolithus* sp.1 by reducing the transportation of Cr from roots to shoots, increasing the N assimilation rates of host through mycelium, and enhancing the available phosphorus (P) and enzyme activities in soil. The above-mentioned process were co-driven by the fungi in Basidiomycetes and Ascomycota phylum, the predominant bacteria *Brevundimonas* sp., *Muribaculaceas*and *Lachnospiraceae*, as well as hexavalent chromium (Cr(VI)) tolerant and reducing strain *Simplicillium* and *Pichia. Pisolithus*sp.2 promoted the Mg accumulation in shoots of host and increased the abundance of *Gibberella*, *Mortierella* and *Didymella* in soil, which accelerated the P solubilization in soil and nutrient transformation in host to response to Cr.

Conclusions Our results showed that *Pisolithus*sp.1 ECM *P. thunbergii* had high adaptability to Cr, and this work also have an important theoretical guiding significance and application value for mycorrhizal phytoremediation of Cr-contaminated soil.

1. Introduction

Chromium (Cr) is one of the top 20 pollutants in the world's recognized list of superhazardous substances (Chrysochoou and Johnston 2012; Dhal et al. 2013). Hexavalent chromium (Cr(VI)) is highly soluble and can enter plants via sulfate transport channels, causing plant metabolism disorders, hindering the absorption of water and nutrients, weakening the photosynthetic rates, and inhibiting the normal growth of plants (Cervantes et al. 2001; Costa 2003; Xu et al. 2021). Cr pollution in soil can also seriously affect human health through food chain and other ways. For example, Cr(VI) could enter the human body mainly through oral ingestion, skin contact and respiratory route, which lead to respiratory system damage, skin ulcer, hematopoietic dysfunction and other diseases (Das et al. 2014; Viti et al. 2014).

Phytoremediation is a process in which plants absorb heavy metals (HMs) or convert valence state of HMs to low toxicity state, and it is easy to be operated and less disturbed in soil structure, and friendly to the environment (Antoniadis et al. 2017; Kaur et al. 2018; Mahar et al. 2016). Ectomycorrhiza is a symbiotic structure formed between ectomycorrhizal fungi (EMF) and higher plant roots (Luo et al. 2014). EMF can improve the drought and HM resistance of host plants (Calvo-Polanco et al. 2019; Liu et al. 2020; Sebastiana et al. 2018), alleviate salt stress injury to plants (Guerrero-Galán et al. 2019), and

enhance the resistance of host plants to pests, pathogenic microorganisms, and other biological stress (Liang et al. 2021; Nerg et al. 2009; Pritsch et al. 2009). The EMF hyphae sheath on plant root surface is the main barrier to prevent HMs from entering the host plants (Lindahl et al. 2007). Johansson et al. (2008) found that inoculation with EMF significantly increased the secretion of oxalic acid (OA), and HM ions could be fixed, thus reducing the toxicity of HMs to host plants. According to Liu et al. (2020), the accumulation of magnesium (Mg), iron (Fe), calcium (Ca), phosphorus (P) and the biomass of host plants were significantly increased after the host plants were inoculated with *Suillus* sp., so as to ensure the normal growth of host plants under Cd stress. Szuba et al. (2017) found that the lead (Pb) content in leaves of ECM plants was lower than that in stems, and the chlorophyll content increased while the carotenoid content remained unchanged, indicating that EMF could protect the photosynthetic organs of host plants from the Pb poisoning.

However, few researches about the environmental impact of mycorrhizal plants on HM contaminated sites. For example, planting *Suillus luteus* mycorrhizal *Pinus massoniana* increased soil water content and reduced soil pH and bulk density (Yu et al. 2020). Liu et al. (2020) found that the symbiosis between *Suillus* sp. and *Pinus sylvetro* could reduce the available concentration of HMs in the soil of tailings ponds and fix the HMs in the soil, thus reducing the toxicity of HMs to host plants. In addition, EMF can interact with the rhizosphere microbial community through a complex network of hyphae. For example, in areas far from roots, the mycelium of ectomycorrhiza will transfer the photosynthate from plants to bacteria that alter the ecology of the soil (Gorka et al. 2019). Fransson et al. (2016) showed that the organic acids (OAs) and unknown compounds secreted by the symbiotes formed by *Pinus sylvestoris* with *Suillus variegatus* or *Piloderma fallax* were the main factors that promoted the growth and diversity of microbial communities. After symbiosis of EMF fungi (*Scleroderma*) with oak (*Quercus palustris*), Uroz et al. (2013) studied the bacterial communities in the rhizosphere and non-rhizosphere soil of ECM plants and found that specific bacterial communities may also affect the enzyme activities in ECM root tips. Therefore, the above studies only focus on the interaction between mycorrhizae and HMs from the one-side of plant, soil or microorganism, and lack a comprehensive explanation.

Our previous study have shown that Cr(VI) tolerant and reducting EMF *Pisolithus* sp.1 (Serial number: KY075875.1) can accelerate the extracellular Cr(VI) reduction by directly secretion of H^+ within 12 days of liquid culture, and can reduce about 75% of Cr (VI) to Cr (III) in the culture system (Shi et al. 2018). In addition, the transcriptome sequencing and comparative transcriptomic analysis of *Pisolithus* sp.1 (Sp1, tolerant to Cr (VI)) and *Pisolithus* sp.2 (Sp2, sensitive to Cr (VI)) before and after Cr(VI) treatment showed that compared with the control, after been treated with 10 mg/L Cr (VI), differentially expressed genes were only significantly enriched in the nitrogen metabolic pathway of Sp1, but not in that of Sp2 (Shi et al. 2020). At the same time, the nitrate reductase (NR) gene (*niaD*) and nitrite reductase (NiR) gene (*niiA*) were significantly up-regulated with Cr(VI) treatment (Shi et al. 2020). However, the physiological and ecological response of ectomycorrhiza and ECM plants to Cr contaminated soil has not been reported. To elucidate this process, this study aimed to investigate: (1) whether Cr could induce self-protection mechanisms driven by nitrogen metabolism in *Pisolithus* sp.1 ECM plants; (2) to investigate the different

physiological mechanism and ecological response of *Pisolithus* sp.1 and *Pisolithus* sp.2 ECM plants responding to Cr. This study provides theoretical basis and application value for the screening of EMF and ECM plants which suitable for mycorrhizal remediation of Cr contaminated soil.

2. Materials and methods

2.1. ECM fungi

Two kinds of ectomycorrhizal (ECM) fungi, Cr(VI) tolerant strain (*Pisolithus* sp.1, Sp1; KY075875) and Cr(VI) sensitive strain (*Pisolithus* sp.2, Sp2; KY075877) were used in our pot experiment (Shi et al. 2020).

2.2. Experimental plant

Pinus thunbergii (*P. thunbergii*) seeds were soaked in 30% H_2O_2 for 10 min for surface disinfection, then rinsed with deionized water, sown in sterilized vermiculite (121℃, 20 min), and incubated in a constant temperature greenhouse (25°C in the morning / 20°C in the evening) for cultivation and seed germination. After 40 days, seedlings with more lateral roots were selected for mycorrhizal synthesis.

2.3. Preparation of ECM Pinus thunbergii

The optimized methods of Sp1 and Sp2 ECM 'Mother' or 'offspring' *P. thunbergii* were based on the methods in Liu et al. (2020) and Shi et al. (2017). The mixed formula of culturing substrate was Kanuma soil: Vermiculite (w/w = 1:1), and the substrate was used after 2h autoclaved at 121 ℃. A ten month old Sp1 or Sp2 ECM 'Mother seedling' were put in the middle of the pot (Top diameter: 15 cm, Height: 10 cm), and then ten *P. thunbergii* seedlings cultivated in 2.2 section were put in pot around the 'Mother seedling'. In the control group, a ten month old non-mycorrhizal (Nm) 'Mother seedling' was substituted for ECM 'Mother seedling' and placed in the middle of the pot. Other processes were the same as the previous description. Four months later, the ECM colonization rates of the offspring *P. thunbergii* seedlings were examined under microscope, and the inoculated seedlings with a ECM rates more than 80% and consistent growth were selected for subsequent pot and acute exposure experiment.

2.4. Pot experiment

The orginal Cr-contaminated soil (CS) and the non-contaminated control soil (NCS) were provided by the research group of Professor Jiyan Shi in Zhejiang University, China. The physicochemical properties of two kinds of soil are shown in Table S1.

For pot experiment, CS and NCS were divided into 12 pots (Top diameter: 15 cm, Height: 10 cm) respectively, and each pot contained 250 g soil. Eighteen consistent ECM (Sp1 or Sp2) and eighteen consistent Nm offspring *P. thunbergii* seedlings were prepared, and three Sp1, Sp2 ECM or Nm *P. thunbergii* seedlings were planted respectively in each pot with CS and NCS. At the same time, the CS and NCS without plants (Np) as control. Therefore, 8 treatments (Sp1 + CS, Sp1 + NCS, Sp2 + CS, Sp2 + NCS, Nm + CS, Nm + NCS, Np + CS, Np + NCS) and 24 samples (8 treatments × 3 repetitions) were set in our experiment. Pots were put in a greenhouse (25°C in the morning / 20°C in the evening) with natural light

and 70% humidity, and 175 mL water was added to each pot weekly. Soil and plant samples were harvested after 5 months.

2.5. Biomass determination

After five months treatment, *P. thunbergii* seedlings were carefully removed from the pots, root system was rinsed with deionized water and soaked in 5 mmol/L EDTA-2Na solution for 30 min, and then rinsed with deionized water for 3 times. The seedlings were divided into aboveground and underground parts and placed in an oven at 70 ℃ for 38 h to dry to constant weight. The dry weight of aboveground and underground were weighed by 1/10,000 balance, and the sum of aboveground and underground biomass was used as the whole plant biomass. After mycorrhiza formation (2.3 section) and before planting in CS or NCS soil, the whole plant biomass of ECM or Nm seedlings were also determinated to calculate the tolerance index (TI) based on the DW of *P. thunbergii* seedlings. TI = (DW of seedlings planting in CS soil - DW of seedlings before planting) / (DW of seedlings planting in NCS soil - DW of seedlings before planting).

2.6. Determination of nutrient elements and Cr concentration in P. thunbergii

The oven dried aboveground and underground of *P. thunbergii* were ground and passed through the 0.15 mm sieve, 0.2450–0.2550 g powder of each sample were put into the electrothermal digestion tube, mixed acid (HNO_3: $HClO_4$ = 87: 13 (v: v)) was added into the tube, and 2.5% HNO_3 were used to dissolve elements and constant volume to 10 mL after digestion. The concentration of P, K, Ca, Mg and Cr in filtered digestion solution were determined by inductively coupled plasma emission spectrometer (ICP-OES, Optima 8000, Shanghai Perkin Elmer, Co., Ltd). Transfer factor (TF) of Cr was calculated as (Cr concentration of aboveground) / (Cr concentration of underground). Another group of crushed samples were weighted in the same way, and then were put into the digestion tube for total N determination. Three grams of K_2SO_4, 0.2 g $CuSO_4$ and 5 mL H_2SO_4 were added in tube, samples were put in the digestion furnace and heated at 420 ℃ for 80 min. After the samples were cooled to room temperature, the content of total N in the solution was determined with Kjeltec 8400 (FOSS).

2.7. Determination of Cr, DTPA-Cr and Cr(VI) in soil

Soil samples were collected from each pot and mixed well. The air-dried soil were ground and passed through the 100 mesh sieve, 0.1950–0.2050 g powder of each soil sample were put into the electrothermal digestion tube, mixed acid (HNO_3: $HClO_4$ = 4:1 (v:v)) was added into the tube, and 2.5% HNO_3 were used to dissolve elements and constant volume to 10 mL after digestion. The concentration of Cr in filtered digestion solution were determined by ICP-OES. Bioconcentration (BCF) was calculated as (Cr concentration of aboveground) / (Cr concentration of soil).

Determination of Diethylene triamine pentaacetic acid extracted Cr (DTPA-Cr) content in soil was refered to Xiong et al. (2018), 4 grams of air-dried soil sample was passed through a 60-mesh sieve, 10 mL DTPA extract solution (including 0.005 mol/L DTPA, 0.01 mol/L $CaCl_2$ and 0.1 mol/L Triethanolamine, pH = 7.3)

was added and samples were shaken for 2h, filtered with 0.45 μm microporous membrane. ICP-OES was used to determine the Cr concentration in the solution. Determination of Cr(VI) content in soil were refered to Jiao (2016), 2.5 g of air-dried soil sample which passed a 60-mesh sieve was weighed and mixed with 25 mL alkaline digestion extract solution, 0.25 mL 0.5 mol/L K_2HPO_4-KH_2PO_4 buffer and 0.2 g $MgCl_2$. The mixed samples were stirred at room temperature for 5 min, and then heated at 90–95 ℃ for 1h. The sample was cooled and filtered with 0.45 μm microporous membrane to collect the filtrate. The pH of filtrate were adjusted to 7.5 with concentrated HNO_3, and then the samples were diluted to 50 mL. Zero point five milliliter of above-mentioned diluted solution were taken and diluted to 25 mL. Zero point five milliliter of concentrated H_2SO_4, 0.5 mL concentrated H_3PO_4 and 2 mL 1,5-Diphenylcarbazide (color developer) were added, mixed and shaken well, then the samples were stood for 10 min, the spectrophotometer was used to measure the absorbance at 540 nm.

2.8. Soil physicochemical properties, enzyme activities and microbial analysis

Please see “Supporting Information”.

2.9. Acute exposure experiment

Acute exposure experiment was set to determinate the movement of NO_3^-, NH_4^+on the root surface of Nm and ECM seedlings by Non-invasive Micro-text Technique (NMT) and also NR activity of roots and coniferous.

The NO_3^- and NH_4^+ motion characteristics was observed by NMT and referred to the methods of Wu et al. (2021). For NO_3^- determination, the basic test solution was 1 mmol/L KCl, 0.1 mmol/L $CaCl_2$ and 0.3 mmol/L MES (pH = 6.0), and the KNO_3 concentration in electrode calibration solution were 0.1 mmol/L and 10 mmol/L respectively. The filling fluid was 10 mmol/L KNO_3.

For NH_4^+ determination, the basic test solution was 1 mmol/L NH_4Cl, 0.1 mmol/L $CaCl_2$ and 0.3 mmol/L MES (pH = 6.0), and the NH_4Cl concentration in electrode calibration solution were 0.1 mmol/L and 10 mmol/L respectively. The filling fluid was 100 mmol/L NH_4Cl.

After the ECM *P. thunbergii* were prepared well (2.3 section), the living Nm seedling roots, and uninoculated/inoculated roots of Sp1 or Sp2 ECM seedlings were fixed in a plastic petri dish which contained the above-mentioned test solution, respectively. For Cr(VI) continuous treatment experiment, after living samples were treated by 100 mg/L Cr(VI) for 96 h, the stable net NO_3^- and NH_4^+ flux were measured respectively. In control group, there was no Cr(VI) pretreating, and 6 replicates were set for each treatment. At the same time, 3 of the 6 plants were taken in each treatment to determinate NR activity of roots and coniferous of Nm and ECM (Sp1 or Sp2) *P. thunbergii* through NR acticity detection kit (Beijing Soleibao BC0080).

2.10. Statistical analysis

One-way analysis of variance (ANOVA), Tukey's honestly significant difference (LSD), and Pearson correlation analysis were conducted using the SPSS 19.0 statistical software. The LSD test was used to explain the significant differences among various treatments at $p < 0.05$. Graph Pad 8.0 was used to draw figures.

3. Results

3.1. Plant growth, nutrition and nitrogen metabolism in mycorrhizal system

After harvest, the growth of ECM *P. thunbergii* seedlings was significantly better than that of Nm seedlings (Fig. 1a), and the dry weight of ECM *P. thunbergii* was also significantly higher than that of Nm seedlings growing in non-contaminated soil (NCS) and Cr contaminated soil (CS). The dry weight of Sp1 ECM *P. thunbergii* growing in CS were significantly higher than Sp2 ECM and Nm *P. thunbergii* (Fig. 1b). In addition, the dry weight of Nm and Sp2 ECM *P. thunbergii* growing in CS were significantly lower than that of Nm and Sp2 ECM *P. thunbergii* growing in NCS, but not Sp1 ECM *P. thunbergii* (Fig. 1b), and the Cr tolerance index (TI) of Sp1 ECM *P. thunbergii* was significantly higher than that of Sp2 ECM *P. thunbergii* and Nm seedlings (Fig. 1c).

There was no significant difference in N content of roots and shoots between ECM and Nm *P. thunbergii* growing in NCS. However, there were significant differences in N content in roots or shoots between ECM and Nm *P. thunbergii* growing in CS. The N content in shoots of Sp1 ECM *P. thunbergii* were significantly higher than those of Sp2 and Nm *P. thunbergii* (Table 1).

Table 1
Nutrient contents in roots and shoots of *Pinus thunbergii*

	Soil		N(mg/g)	P(mg/g)	K(mg/g)	Ca(mg/g)	Mg(mg/g)
Root	NCS	Nm	5.72 ± 0.01 c	0.46 ± 0.02 c	4.49 ± 0.79 a	4.27 ± 0.54 c	1.70 ± 0.24 cd
		Sp1	5.76 ± 0.39 c	0.93 ± 0.08 ab	3.87 ± 0.74 a	2.97 ± 0.17 d	2.84 ± 0.59 b
		Sp2	5.31 ± 0.46 c	1.00 ± 0.02 ab	4.27 ± 0.41 a	4.64 ± 0.57 bc	4.39 ± 0.52 a
	CS	Nm	7.94 ± 0.91 b	0.56 ± 0.15 c	3.72 ± 0.74 a	6.19 ± 0.93 a	1.06 ± 0.13 d
		Sp1	9.55 ± 1.02 a	1.10 ± 0.10 a	4.03 ± 0.09 a	5.64 ± 0.67 ab	1.49 ± 0.03 cd
		Sp2	8.79 ± 0.93 ab	0.83 ± 0.13 b	3.88 ± 0.64 a	4.10 ± 0.78 cd	2.09 ± 0.06 c
Shoot	NCS	Nm	5.01 ± 0.66 c	0.53 ± 0.08 b	3.36 ± 0.27 abc	7.66 ± 0.15 b	1.13 ± 0.17 c
		Sp1	5.84 ± 0.11 c	0.54 ± 0.03 b	2.05 ± 0.14 d	4.23 ± 0.44 d	1.74 ± 0.16 b
		Sp2	6.38 ± 0.45 c	0.59 ± 0.01 b	2.79 ± 0.38 bc	6.91 ± 0.63 bc	1.76 ± 0.37 b
	CS	Nm	5.82 ± 0.85 c	0.63 ± 0.17 b	3.64 ± 0.67 a	9.46 ± 0.54 a	1.25 ± 0.03 c
		Sp1	15.49 ± 1.29 a	0.96 ± 0.10a	3.45 ± 0.50 ab	6.01 ± 0.50 c	1.85 ± 0.16 b
		Sp2	10.42 ± 0.59 b	0.96 ± 0.09 a	2.74 ± 0.22 c	5.97 ± 0.85 c	2.74 ± 0.26 a

NCS, Non-contaminated soil; CS, Cr contaminated soil. Data are means ± standard error of 3 replicates. Different letters in the same element of root or shoot group indicate significant differences by Tukey's test ($p < 0.05$).

The P content in roots of ECM *P. thunbergii* were significantly higher than that of Nm *P. thunbergii* under CS and NCS conditions. In addition, the P content in roots of Sp1 ECM *P. thunbergii* were significantly higher than that of Sp2 ECM and Nm seedlings growing in CS. On the other hand, there was no significant difference in P content of shoots between ECM and Nm *P. thunbergii* growing in NCS. The P content in shoots of ECM *P. thunbergii* were significantly higher than that of Nm *P. thunbergii*, but there was no significant difference in P content of shoots between Sp1 and Sp2 ECM *P. thunbergii* growing in CS soil (Table 1).

The Mg content in roots or shoots of ECM *P. thunbergii* were significantly higher than that of Nm *P. thunbergii* in all treatments. In addition, Mg content in roots of Sp2 ECM *P. thunbergii* which growing in NCS, and shoots of Sp2 ECM *P. thunbergii* which growing in CS were significantly higher than those of Sp1 ECM and Nm *P. thunbergii* (Table 1).

There was no significant difference in the net NO_3^- flux on root surface among different treatments without Cr(VI) pretreating (control), and the order of net NO_3^- flux on root surface were Sp1-ECM root > Sp2-ECM root > Sp2-Nm root > Sp1-Nm root > Nm root after Cr(VI) pretreating (Cr(VI)) (Fig. 2a). The net NH_4^+ flux on surface of Sp2-ECM root was significantly higher than other roots in control group, but no significant differences were found among all roots in Cr(VI) group (Fig. 2b). In addition, Cr(VI) significantly increased the NO_3^- absorption rate of Sp1-Nm, Sp1-ECM, Sp2-Nm and Sp2-ECM roots (Fig. 2a), and significantly reduced the NH_4^+ absorption rate of various roots (Fig. 2b).

There was no significant differences in NR activity of five kinds of roots without Cr(VI) pretreating. The NR activity order of five kinds of roots with Cr(VI) pretreating were Sp1-ECM root > Sp2-ECM root > Sp1-Nm root > Sp2-Nm root > Nm root (Fig. 2c). The NR activity of coniferous of three kinds of *P. thunbergii* without Cr(VI) pretreating showed no significant differences, and the NR activity of coniferous of Sp1 ECM *P. thunbergii* were significantly higher than those of Nm and Sp2 ECM *P. thunbergii* with Cr(VI) pretreating (Fig. 2d). In addition, the NR activity of Sp1- and Sp2- ECM roots with Cr(VI) pretreating were significantly higher than that of Sp1- and Sp2- ECM roots without Cr(VI) pretreating, but not the same for other roots (Fig. 2c). The NR activity in coniferous of Sp1 ECM seedlings with Cr(VI) pretreating significantly higher than that of Sp1 ECM seedlings without Cr(VI) pretreating, but not the same for the NR activity in coniferous of Nm and Sp2 ECM seedlings (Fig. 2d).

3.2. Analysis of nutrients, physicochemical properties, Cr content and morphology in soil

In NCS soil, both ECM *P. thunbergii* and Nm *P. thunbergii* can significantly reduce the content of NO_3^--N and NH_4^+-N compared with Np treatment (Fig. 3a, 3b). In addition, the available P content of NCS was significantly increased by planting Sp1 and Sp2 ECM *P. thunbergii* compared with Np and Nm treatments (Fig. 3c). In CS soil, Sp1 ECM *P. thunbergii* significantly decreased NO_3^--N content and increased available P content compared with other treatments. In addition, compared with other treatments, Sp2 ECM *P. thunbergii* had the best improvement effect on soil available K (Fig. 3d). For each treatment, NO_3^--N, NH_4^+-N, available P and available K of *P. thunbergii* growing in CS were significantly higher than those of NCS.

In NCS soil, Sp2 ECM *P. thunbergii* significantly increased the activity of soil urease (U) and alkaline phosphatase (Alk) compared with Sp1 ECM and Nm treatments. Sp1 and Sp2 ECM *P. thunbergii* significantly increased the activity of acid phosphatase (Aci) and Alk compared with Nm *P. thunbergii*. In

CS soil, planting Sp1 ECM *P. thunbergii* significantly increased the activity of U and Aci compared with Sp2 ECM and Nm *P. thunbergii* (Fig. S2).

In NCS soil, both Nm and ECM *P. thunbergii* significantly reduced soil pH compared with Np treatment, and ECM seedlings had the most obvious reduction effect. In CS soil, Sp1 ECM *P. thunbergii* significantly reduced soil pH compared with Np and Nm treatments (Fig. S2d). In CS soil, Sp1 and Sp2 ECM seedlings significantly increased the EC compared with Np and Nm treatments (Fig. S2e).

After harvest, the Cr concentration in ECM *P. thunbergii* were significantly higher than that of Nm seedlings. In addition, the Cr concentration in shoots of Sp2 ECM *P. thunbergii* were significantly higher than that of Sp1 ECM *P. thunbergii*, but there was no significant change of Cr concentration in roots of Sp1 and Sp2 ECM *P. thunbergii* (Fig. 4a, 4b). The Cr content per shoot or root in ECM *P. thunbergii* were significantly higher than that of Nm seedlings. However, there were also no significant difference of Cr content per shoot or root between Sp1 and Sp2 ECM *P. thunbergii* (Fig. S3a, S3b).

The transfer factor (TF) and bioconcentration factor (BCF) of ECM *P. thunbergii* to Cr were significantly higher than that of Nm *P. thunbergii*. In addition, the TF and BCF of Sp2 ECM *P. thunbergii* to Cr were significantly higher than that of Sp1 ECM and Nm *P. thunbergii* (Fig. 4c, 4d).

Both Nm and ECM *P. thunbergii* can significantly reduce the soil Cr(VI) and DTPA-Cr concentration in Cr contaminated soil compared with Np treatment, and Sp1 ECM *P. thunbergii* significantly reduced the Cr(VI) and DTPA-Cr concentration compared with other treatments (Fig. 5).

3.3. Abundance and structural analysis of soil microbial communities

Compared with Np treatment, Nm seedlings significantly decreased the diversity (Shannon, Simpson) of bacterial community, but increased the diversity of fungal community. Compared with Nm treatments, Sp1 and Sp2 ECM seedlings increased the diversity index of bacterial community, and Sp2 ECM seedlings significantly increased the richness index (Chao1) compared with Nm seedlings. However, there were no significant differences among Nm, Sp1 and Sp2 ECM treatments on fungal richness and diversity, but Sp1 treatment had the largest contribution to fungal community diversity (Table S2). The β-diversity principal coordinate analysis (PCoA) of the microbial community structure are shown in Fig. S4. Among them, 30.8% (PCoA-X) and 19% (PCoA-Y) of the bacterial data explained the total variation, and 14.2% (PCoA-X) and 12.3% (PCoA-Y) of the fungal data explained the total variation.

The results of Heatmap analyses of top 20 abundant bacteria and fungi species in each group at the genus level after planting Nm and ECM seedlings showed that the soil appeared the obvious separation and clustering in the community structure of bacteria and fungi respectively (Fig. 6). The bacterial community composition in the soils with Sp1 and Sp2 ECM treatments were similar compared with Np and Nm treatments (Fig. 6a). Compared with Np treatment, Nm treatment significantly increased the relative abundance of *Akkermansia*, L*leibacterium*, *Bifidobacterium, Lactobacillus*, while significantly

reduced the relative abundance of *Ruminiclostridium_9*, *Sphingomonas*, *Parabacteroides, Pelomonas, Vibrionimonas, Brevundimonas, Lachnospiraceae, Aquabacterium, Dubosiella, Blautis, Faecalibaculum* and *Coriobacteriaceae* in soil. Sp1 and Sp2 treatments significantly increased the relative abundance of *Allorhizobium, Sphingomonas, Parabacteroides, Pelomonas, Vibrionimonas, Brevundimonas* and *Lachnospiraceae*, while significantly reduced the relative abundance of *Aquabacterium*, *Dubosiella*, *Blautia*, *Lactobacillus*, *Faecalibaculum* and *Coriobacteriaceae* compared with Np treatment. The relative abundance of *Muribaculaceas*, *Brevundimonas* and *Lachnospiraceae_NK4A136* in Sp1 treatment were significantly higher than other three treatments, and *Alistipes*, *Rikenellaceae, Ruminclostridium*, *Parabacteroides*, *Pelomonas* in Sp2 treatment were also significantly higher than other three treatments (Fig. 6a).

The results of Heatmap analyses of top 20 top abundant fungal species in each group at the genus level showed that the fungal structures treated by Np and Sp2 were similar to those treated by Nm and Sp1. Compared with Np treatment, Nm treatment significantly increased the relative abundance of *Thermoascus*, *Mycosphaerella, Lecanicillium, Xeromyces* and *Sarocladium*, while decreased the relative abundance of *Mortierella*, *Didymella*, *Malassezia*, *Phialemoniopsis*, *Pseudogymnoascus*, *Candida*, *Acremonium*, *Pichia*, *Simplicillium*, *Cutaneotrichosporon.* Sp1 treatment significantly increased the relative abundance of *Aspergillus, Candida, Acremonium, Fusarium, Pichia*, *Tausonia*, *Simplicillium*, *Cutaneotrichosporon*, *Cenococcum* and *Mycosphaerella*, while Sp2 treatment significantly increased the relative abundance of *Gibberella*, *Mortierella*, *Didymella* and *Fusarium* compared with the Nm and Np treatments. Nm, Sp1 and Sp2 treatment significantly reduced the relative abundance of *Malassezia*, *Phialemoniopsis* and *Pseudogymnoascus* in soil compared with Np treatment (Fig. 6b).

3.4. Principal component analysis and correlation analysis of physiological and ecological indicators

The results of principal component analysis (PCA) showed that Sp1 inoculation made a major contribution to increase the growth and Cr tolerance, net NO_3^- flux on surface of root, N concentration in root and shoot, P and K concentration in root, NR activity in coniferous of *P. thunbergii*, EC, enzyme activity (Urease, acid phosphatase (Aci), alkaline phosphatase (Alk)), shannon (FShan) and simpson (FSim) of fungi, as well as the relative abundance of microbial (*Brevundimonas* (Bre), *Simplicillium* (Sim), *Pichia* (Pic), *Cutaneotrichosporon* (Cut), *Aspergillus* (Asp) and *Muribaculaceae* (Mur)) in Cr contaminated soil. In addition, Sp1 inoculation also made contribution to the reduction of pH, NO_3-N, DTPA-Cr and Cr(VI) concentration in soil.

On the other hand, inoculation with Sp2 made a major contribution to increase available K, Mg concentration of root and shoot, transport factor (TF), Bioconcentration factor (BCF), Cr concentration in shoot (SCr) and root (RCr), total accumulated Cr in shoot (SACr), P concentration in shoot (SP), Chao 1 of bacteria (BChao1), shannon and simpson of bacteria (BShan, BSim), and the relative abundance of microbial (Pleomonas (Ple), Vibrionimonas (Vib), Lachnospiraceae (Lac), and Fusarium (Fus)) were also

increased. In addition, Sp2 inoculation also made contribution to decrease K concentration in shoot, Ca concentration in shoot and root, and the net NH_4^+-N flux (NH_4^+-NF) on surface of root.

Pearson correlation coefficient (Pearson's r) usually measures the relationship between data sets by testing the linear relationship between interval variables. The closer the correlation coefficient is to 1 or -1, the stronger the correlation is. By contrast, the closer the correlation coefficient is to 0, the weaker the relationship.

The TI had significant positive correlations with RACr ($p < 0.01$), N concentration in root had significant positive correlations with K concentration in root and urease (U) activity in soil ($p < 0.01$). P concentration in root had significant positive correlations with acid phosphatase (Aci) and alkaline phosphatase (Alk) in soil ($p < 0.01$). K concentration in root had significant positive correlations with N concentration in root and U activity in soil ($p < 0.01$). N concentration in shoot had significant positive correlations with available P concentration in soil ($p < 0.01$). P concentration in shoot had significant negative correlations with Ca concentration in shoot ($p < 0.01$). Ca concentration in shoot had significant negative correlations with P concentration in shoot and the abundance of *Vibrionimonas* (Vib). Mg concentration in shoot had significant positive correlations with available K in soil ($p < 0.01$). NR activity of root and coniferous had significant positive correlations with the abundance of *Mycosphaerella* (Myc) and total accumulated Cr in root respectively ($p < 0.01$). Net NO_3^-- flux (NNOF) had significant positive correlations with dry weight ($p < 0.05$). NO_3^--N concentration in soil had significant positive correlations with *Pichia* (Pic) and *Simplicillium* (Sim) ($p < 0.01$). pH in soil had negative correlations with dry weight, TI and the abundance of *Brevundimonas* (Bre) ($p < 0.05$). Total accumulated Cr in shoot had significant positive correlations with EC and Cr concentration in root ($p < 0.01$). Cr(VI) concentration in soil had significant positive correlations with NH_4^+-N concentration in soil ($p < 0.05$). DTPA-Cr had significant positive correlations with NO_3^--N and NH_4^+-N concentration in soil ($p < 0.05$) and negative correlations with NR activity in coniferous ($p < 0.01$). Chao1 of bicteria had significant positive correlations with Mg concentration in root and shoot, and available K in soil ($p < 0.05$). Shannon and Simpson of bacteria had significantly positive correlations with net NH_4^+ flux, EC, SACr and the abundance of Ple ($p < 0.05$). Shannon of fungi had significant positive correlations with TI, RN, RP, RK, U, Alk, RACr and Bre ($p < 0.05$).

4. Discussion

4.1. The important factors affecting the growth and Cr tolerance of P. thunbergii

Ectomycorrhizal fungi (EMF) can help host plant absorb water and mineral elements, improve their survival rates and growth rates, and also help the host plant survive in a variety of harsh and barren environments such as pests, diseases, salt stress, HM stress and drought stress (Pena et al. 2014; Pritsch et al. 2009). In this study, inoculation with Cr(VI) tolerant EMF Sp1 significantly increased the growth and Cr tolerance of *P. thunbergii* (Fig. 1). Dry weight (DW) were significantly ($p < 0.05$) negative correlated with

soil pH but significantly ($p < 0.01$) positive correlated with the absolute value of net NO_3^- flux (NNOF) and abundance of *Brevundimonas* sp. ($p < 0.05$) (Fig. 2a, 8). In addition, Cr tolerance index (TI) had significantly positive correlations with total accumulated Cr in root ($p < 0.01$), N and K concentration ($p < 0.05$) in root, and urease (U) activity, Shannon of fungi, the abundance of *Brevundimonas* sp. ($p < 0.05$) in soil, but also had significantly ($p < 0.05$) negative correlations with soil pH (Fig. 8). Therefore, the relationship among soil pH, fungal diversity, dominant bacteria *Brevundimonas* sp., soil enzyme activity and N metabolism was an important factor to affect growth and Cr tolerance of *P. thunbergii.*

4.2. Microbial mechanisms of mycorrhizal plants responding to Cr

The physicochemical properties of soil were considered to be the main reason of microbial communities richness and diversity in the plant rhizosphere (Reverchon et al. 2012). In this study, planting Sp1 ECM *P. thunbergii* significantly reduced soil pH compared with Np and Nm treatment (Fig. S2d). In our previous study, we found that Cr(VI) tolerant EMF Sp1 can secrete hydrogen ions (H^+) and OA to decrease the environment pH and play an important role in Cr(VI) reduction (Shi et al. 2018). On the other hand, OA directly secreted by *Suillus variegatus* inoculated *Piloderma fallax* was the main factor that increased the growth and diversity of microbial communities (Fransson et al. 2016). A certain bacterial communities may also determine the enzyme activity of ECM root tips (Uroz et al. 2013). Therefore, planting Sp1 and Sp2 ECM *P. thunbergii* also increased the bacterial richness and diversity, fungal diversity, and the abundance of dominant bacteria and fungi group in our study (Fig. 6; Table S2). Ascomycetes and basidiomycetes, the two main phylum of ECM fungi, are also the main decomposers of fungi, which are usually found in heavy metal polluted environments and are considered to be more adaptable to adversity (Shi et al. 2019). Ascomycota are mostly saprophytes, which play an important role in degrading soil organic matter, and Basidiomycetes can decompose lignocellulose in plant residues (Jiang et al. 2021). Sp1 inoculation significantly increased the proportion of Ascomycota in all fungal phyla compared with other treatments (Fig. S5b). In addition, the abundance of *Aspergillus*, *Candida*, *Acremonium*, *Fusarium*, *Pichia*, *Tausonia*, *Simplicillium*, *Cutaneotrichosporon*, *Cenococcum* and *Mycosphaerella* genus all significantly increased after planting Sp1 ECM *P. thunbergii* (Fig. 6b). Gong et al. (2021) found that *Candida* can be used to treat phenol-contaminated wastewater and soil. *Aspergillus* and *Pichia* have also been reported to reduce Cr(VI) and detoxify (Park et al. 2005; Ksheminska et al. 2008). Sp2 ECM seedlings significantly increased the relative abundance of *Gibberella*, *Mortierella* and *Didymella* in our study. Tu et al. (2018) found that *Gibberella* showed high tolerance to acidic environment and Cu pollution. *Mortierella* has the potential to dissolve soil phosphorus by releasing OA in different soils (Osorio et al. 2014). Li et al. (2018) also found that *Mortierella* plays a key role in soil nutrient transformation and promotion of plant growth.

Brevundimonas sp., as a P solubilizing and mobilizing bacteria significantly positively correlated with growth and Cr tolerance of *P. thunbergii* (Fig. 8), also has the function of plant growth promoting, and copper (Cu), arsenic (As), lead (Pb), chromium (Cr) and mercury (Hg) tolerance (Kour et al. 2021; Manohari et al. 2021; Sforza et al. 2018; Singh et al. 2021). Take *Brevundimonas diminuta* MYS6 as an

example. When Cu was exposed, its EPS increased by 18.6%, indicating that it played an internal defense role under Cu stress. The root and stem length, dry and fresh biomass and leaf chlorophyll of *Helianthus annuus* increased by 1.5, 1.7, 9.9, 15.8 and 2.1 times, respectively (Manohari et al. 2021). Singh et al. (2016) found that the *Oryza sativa* growth promoting traits of *Brevundimonas* sp. revealed the inherent ability of siderophores, phosphate solubilization, indole acetic acid (IAA), 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase production which may be associated with increased biomass, chlorophyll and MDA content of rice and thereby promoting plant growth, and also significantly restored the hampered root epidermal and cortical cell growth of rice plant and root hair elimination.

In addition, soil enzyme activity can be used as an indicator of soil microbial functional diversity (Sanchez-Hernandez et al. 2017). U can involve in the organic nitrogen cycle in the soil, making N available to plants (Zhang et al. 2018). Soil phosphatase is an index for evaluating the direction and intensity of phosphorus (P) biotransformation in the soil because it can catalyze the mineralization of organic P in the soil, and its activity directly affects the decomposition, transformation and bioavailability of organic P in the soil (Awuah et al. 2019). Some mycorrhizal fungi have phosphatases and phytases that mobilize large amounts of organophosphorus (Summerbell et al. 2005). Liu et al. (2020) found that planting ECM *P. sylvestris* in soil of lead (Pb)-zinc (Zn)-cadmium (Cd) tailings ponds can significantly increase soil U and phosphatase activity, as well as the P accumulation in shoot of ECM *P. sylvestris* compared with Nm seedlings (Liu et al. 2020). Even though we did not check the concentration of available nitrogen (N) in soil, we could see the N concentration in root and shoot have significantly positive correlations with U, acid phosphatase (Aci) and alkaline phosphatase (Alk) (Fig. 8). Therefore, U and phosphatase activity were increased by EMF and *Simplicillium* after soil planted with Sp1 ECM *P. sylvestris.* The results directly lead to an increase in available N, P in soil and an increase in N, P concentration in shoot and root of Sp1 ECM *P. sylvestris* in our study (Fig. 3c, 8, S2; Table 1).

4.3. Nitrogen metabolism mechanisms of mycorrhizal plants in response to Cr

For N metabolism, plants usually use NO_3^--N and NH_4^+-N and then convert these to various amino acids (Giagnonia et al. 2016). In our study, DW of *P. thunbergii* planted in Cr contaminated soil were significantly ($p < 0.05$) positive correlations with the absolute value of net NO_3^- flux (NNOF) (Fig. 8), and inoculation with Sp1 significantly increased the NNOF on the surface of root (Fig. 2a), nitrate reductase (NR) activity of roots (Fig. 2c) and coniferous (Fig. 2d), and also N concentration in shoot (Table 1), but decreased the NO_3^--N concentration in soil compared with Nm and Sp2 ECM *P. thunbergii* (Fig. 3a). Therefore, Sp1 ECM *P. thunbergii* assimilated more NO_3^--N than Sp2 ECM seedlings. However, many articles found that heavy metals were harmful to N assimilation and reduce the activity of related enzymes. For example, Shahid et al. (2018) found that Cr-stress induced significant reductions in NO_3^-, and NO_2^- contents in leaves of 2x- and 4x-grafted *Kinnow mandarins* and decreased the activities of NR, nitrite reductase (NiR), glutamine synthetase (GS) and glutamate synthetase (GOGAT). Singh et al. (2019) found that NR activity in plants was also inhibited by Cd toxicity. In addition, the low nitrate concentration

in the leaves showed that nitrate uptake by the roots was decreased by Cr toxicity (Singh et al. 2013). Indeed, Cr inhibited the net NO_3^- (a) and NH_4^+(b) flux on the root surface (within 200 μm), and also decreased the NR activity in roots of NM *P. thunbergii*, but it had the opposite effect on ECM seedlings (Fig. 2). Cr toxicity to Sp1 ECM *P. thunbergii* was lower than Sp2 ECM *P. thunbergii*, because of the higher TI (Fig. 1) and lower Cr concentration in shoot, transfer factor (TF), bioconcentration factor (BCF) of Sp1 ECM *P. thunbergii* (Fig. 4). However, even though Cr concentration in shoot and root, TF and BCF of Nm *P. thunbergii* were significantly lower than ECM *P. thunbergii* (Fig. 4), but the TI of Sp1 ECM *P. thunbergii* was also significantly higher than Nm plants (Fig. 1, Fig. 2). On the other hand, NR can act as an important enzyme for nitric oxide (NO) production (Singh et al. 2019). As a signaling molecule, NO plays an important role in many plant processes such as programmed cell death, stomatal closure, growth, germination, signaling, biotic and abiotic stress response (Farnese et al. 2016; Sanz-Luque et al. 2015). For example, NO can eliminate Cr induced ROS by improving the antioxidant capacity of plants, thereby maintaining the stability of the plant cell environment (Asgher et al. 2018; Correa-Aragunde et al. 2015).

Planting two kinds of ECM *P. thunbergii* could significantly reduce the concentration of DTPA-Cr and Cr(VI) in Cr-contaminated soil, and the Sp1 ECM *P. thunbergii* had a significant better reducing effect compared with other treatments (Fig. 5). Ray and Adholeya (2009) found that ECM fungi secreted a large amount of OA in mycorrhizal symbionts to chelate HMs, thereby reducing the available concentration of HMs. In addition, Sp1 strain also had function of Cr(VI) reduction in our previous study (Shi et al. 2018). We also found that DTPA-Cr concentration in Cr-contaminated soil had a significantly ($p < 0.05$) positive correlation with *Pichia* and *Simplicillium* (Fig. 8). *Pichia jadinii* and *Pichia anomala* could reduce Cr(VI) and *Pichia guilliermondii* could adsorb Cr(VI) and Cr(III) (Ksheminska et al. 2003; Martorell et al. 2012). *Simplicillium chinense* can also be used for Cd and Pb biosorption and to enhance phytoremediation of heavy metals in soils (Jin et al. 2019; 2020).

5. Conclusions

Inoculation with *Pisolithus* sp.1 significantly increased the growth and Cr tolerance of *P. thunbergii* by promoting the N, P transform in soil and accelerated NO_3^--N inflow from surface of Sp1 ECM roots and increased the activity of nitrate reductase activity in roots and needles. The decrease of soil pH after inoculation with Sp1 may be related to the OA secretion by Sp1, which promoted the fungi diversity of Ascomycetes and Basidiomycetes phylum with the function of P and N activation, Cr(VI) reduction and detoxify function, and also increased the relative abundance of the dominant bacteria *Brevundimonas* sp., a kind of P solubilization and plant growth promoting bacteria. In addition, the reduction of Cr(VI) and DTPA-Cr concentration in soil may due to the contribution of Cr(VI) reducting strain Sp1, and *Simplicillium*, *Pichia* respectively. *Pisolithus* sp.2 promoted the Mg accumulation in shoots of host and abundance of *Gibberella*, *Mortierella* and *Didymella* in soil, which may accelerate the dissolution of P in soil and the nutrient conversion of Cr by host plants.

Declarations

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Author contributions

Liang Shi: Conceptualization, Data curation, Writing-original draft. **Binhao Liu:** Formal analysis. **Xinzhe Zhang:** Formal analysis and Data curation. **Xuan Zhao:** Data curation. **Zhenguo Shen:** Review, Supervision, Conceptualization. **Yahua Chen:** Supervision, Conceptualization, Review and editing.

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Declaration of competing interest

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

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Figures

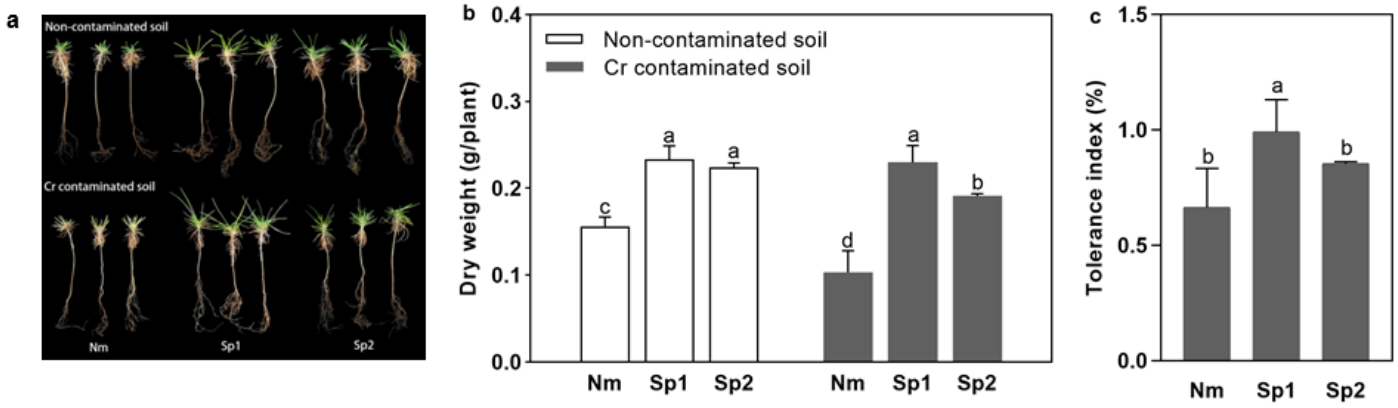


Figure 1

The growth (a), dry weight (b) and Cr tolerance index (c) of Nm and ECM (*Pisolithus* sp. 1 (Sp1) and *Pisolithus* sp. 2 (Sp2)) *Pinus thunbergii* grown in non-contaminated soil or Cr contaminated soil for 5 months. Data are means ± SD of 3 biological replicates. Different letters in each figure indicate significant ($p < 0.05$) differences according to Tukey's test.

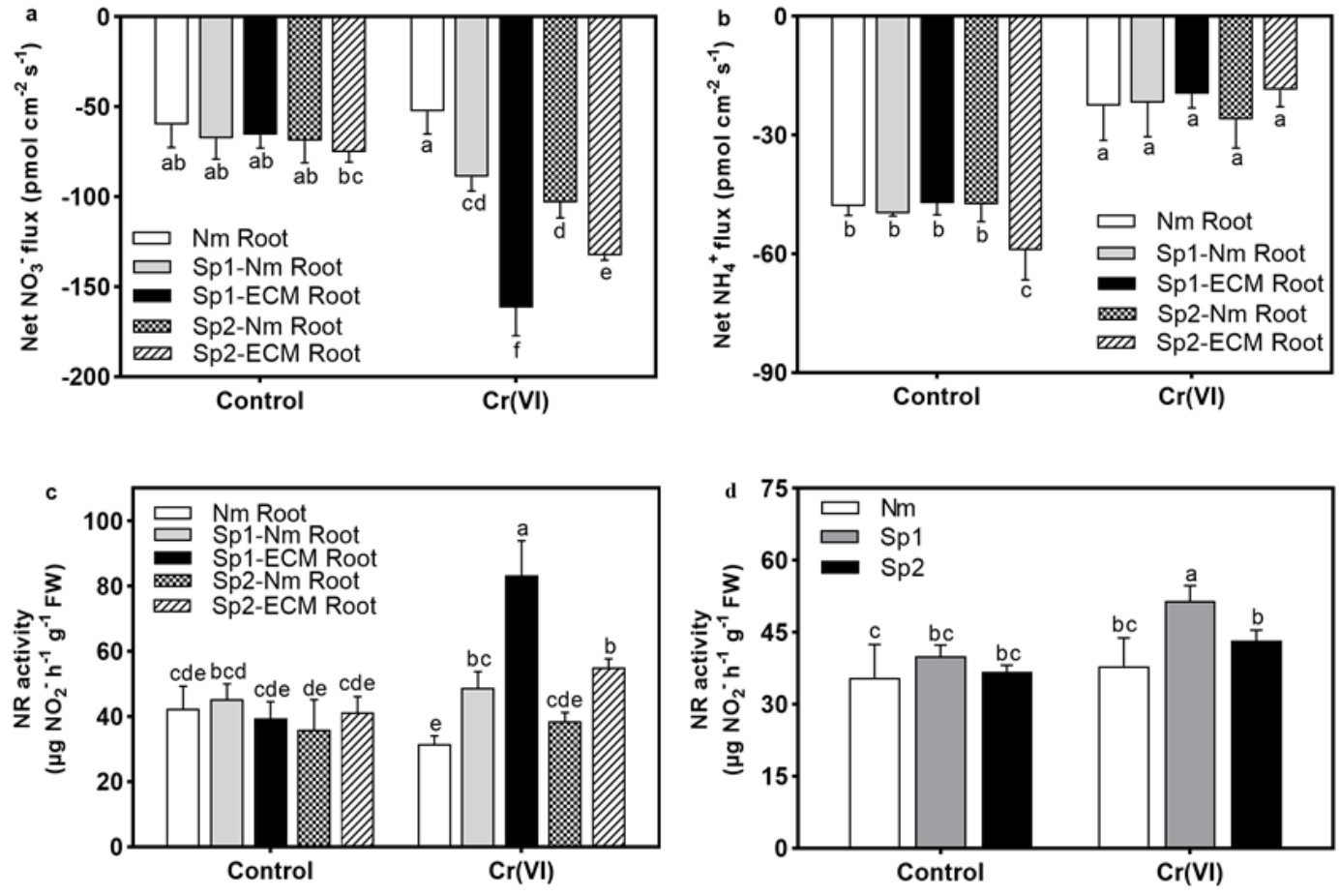
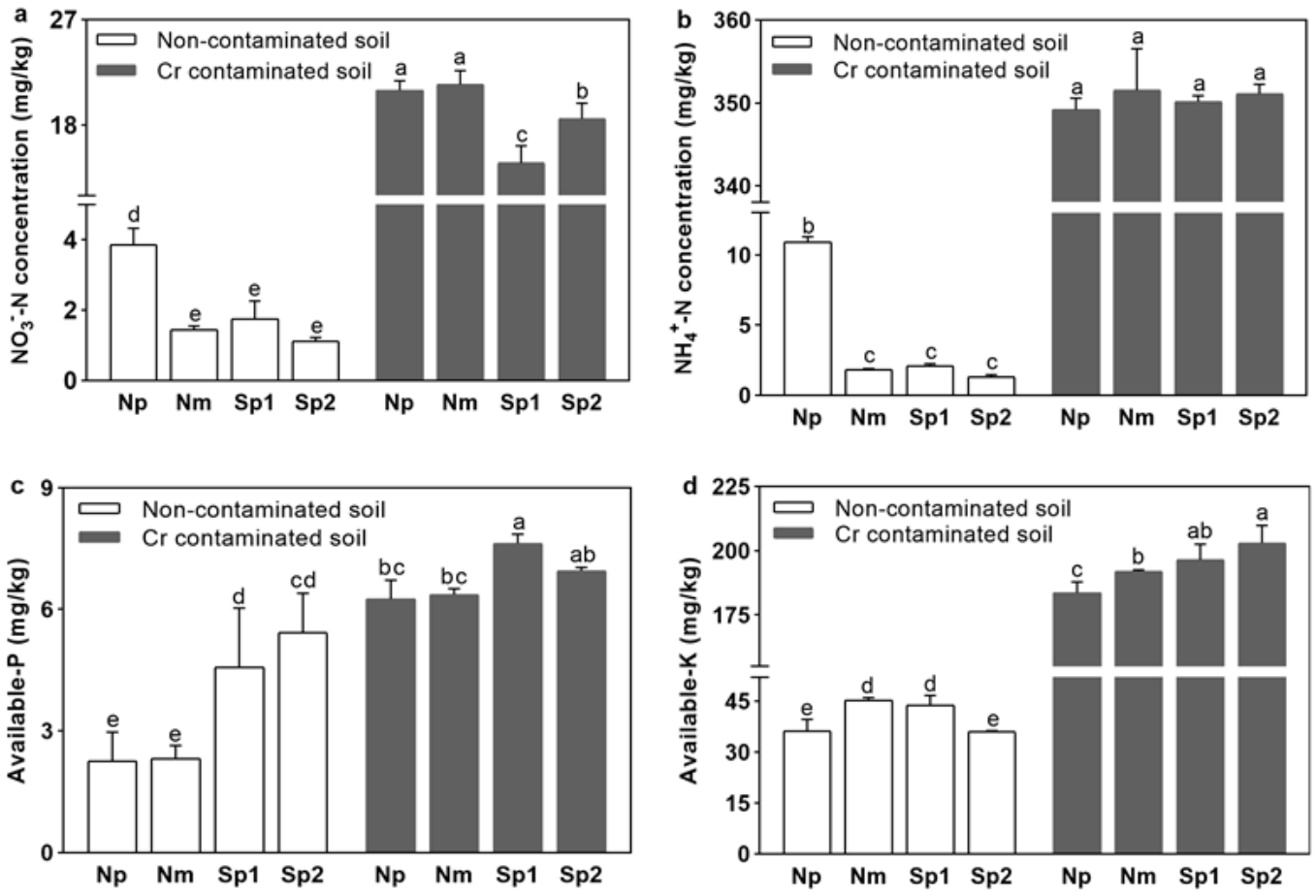


Figure 2



Effect of Cr(VI) on the net NO_3^- (a) or NH_4^+(b) flux on the surface (within 200 μm) of roots, and nitrate reductase (NR) activity of roots (c) and coniferous (d) of Nm and ECM (Sp1 or Sp2) *Pinus thunbergii*. Nm root, uninoculated roots of Sp1 ECM seedlings (Sp1-Nm), inoculated roots of Sp1 ECM seedlings (Sp1-ECM), uninoculated roots of Sp2 ECM seedlings (Sp2-Nm) and inoculated roots of Sp2 ECM seedlings (Sp2-ECM). 100 mg/L Cr(VI) continuously treated for 96 h, stabilizing net $NO_3^-$and NH_4^+ flux were measured respectively. Control group without Cr(VI) pretreating and three replicates were set for each treatment. Data are means ± SD of three biological replicates and different letters in each figure indicate significant ($p < 0.05$) differences according to Tukey's test.

Figure 3

NO_3^--N (a), NH_4^+-N (b), available-P (c), available-K (d) in soil after ECM (Sp1, Sp2) and Nm *Pinus thunbergii* grown for 5 months (Np, no plant). Data are means ± SD of 3 biological replicates. Different letters in each figure indicating significant ($p < 0.05$) differences according to Tukey's test.

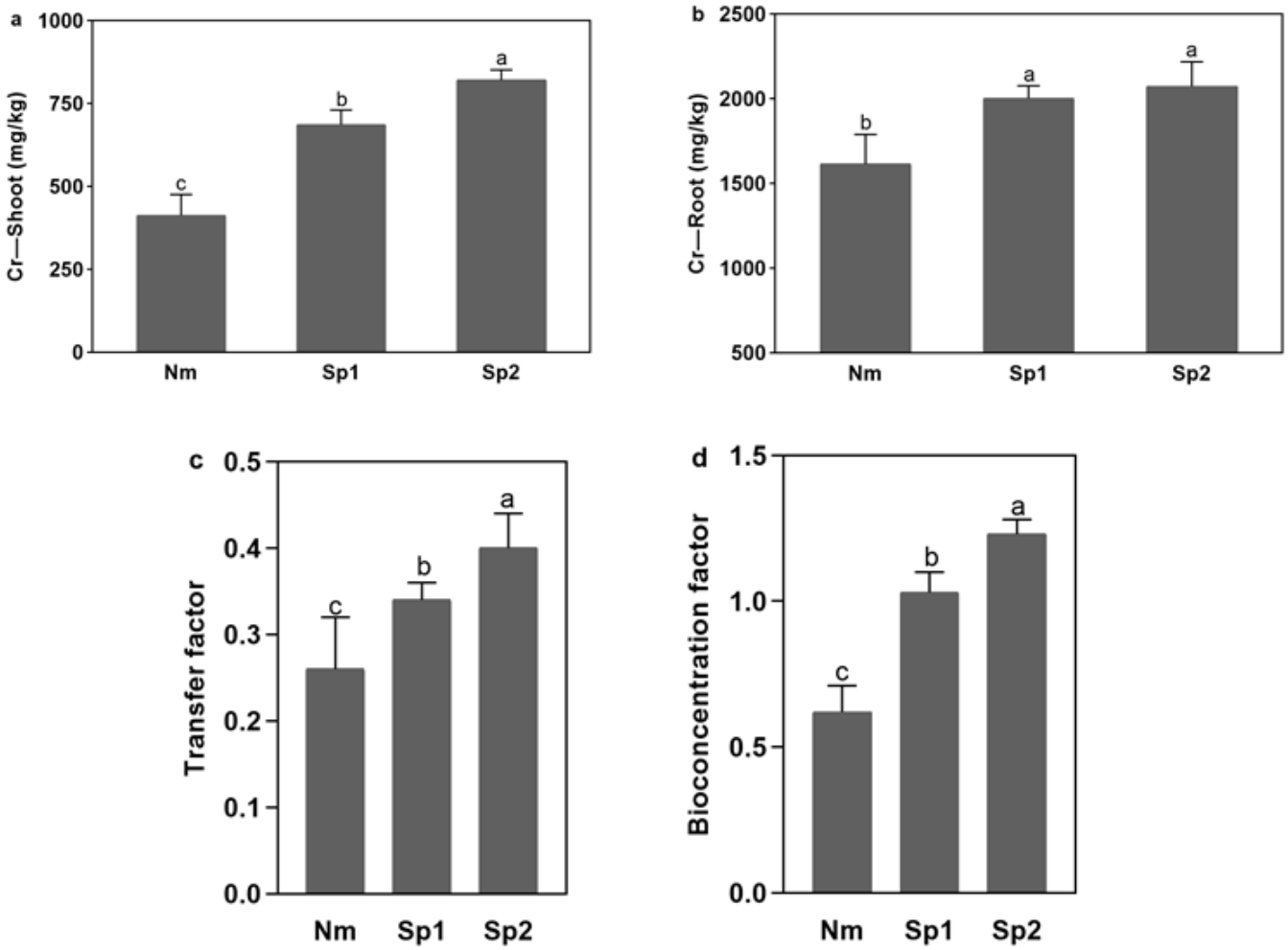


Figure 4

Cr concentration in shoot (a) and root (b), and Cr transfer factor (c) and bioconcentration factor (d) of ECM (Sp1, Sp2) and Nm *Pinus thunbergii* after grown in Cr contaminated soil for 5 months. Data are means ± SD of 3 biological replicates. Different letters in each figure indicating significant ($p < 0.05$) differences according to Tukey's test.

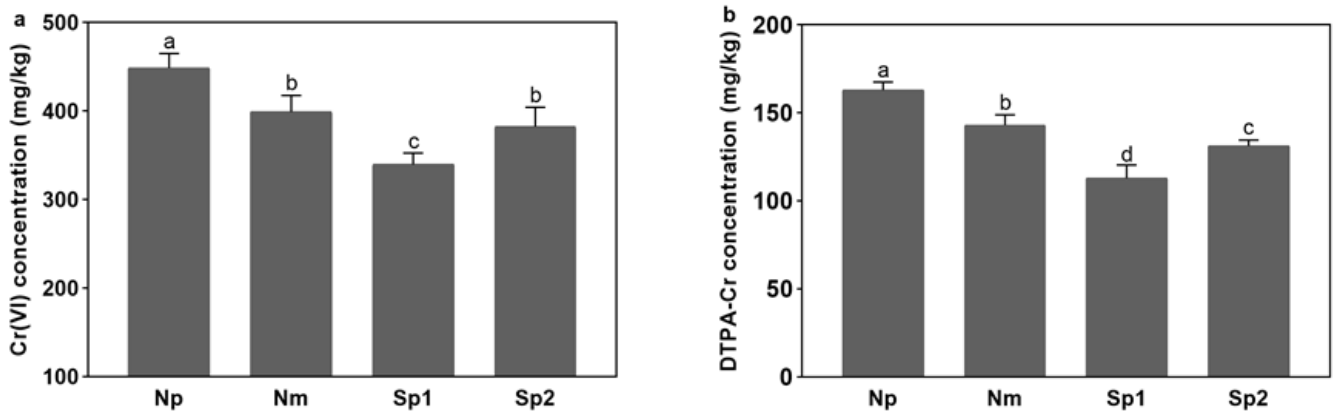


Figure 5

Concentration of Cr(VI) (a) and DTPA-Cr (b) in Cr contaminated soil after ECM (Sp1, Sp2) and Nm *Pinus thunbergii* grown for 5 months (Np, no plant). Data are means ± SD of 3 biological replicates. Different letters in each figure indicating significant ($p < 0.05$) differences according to Tukey's test.

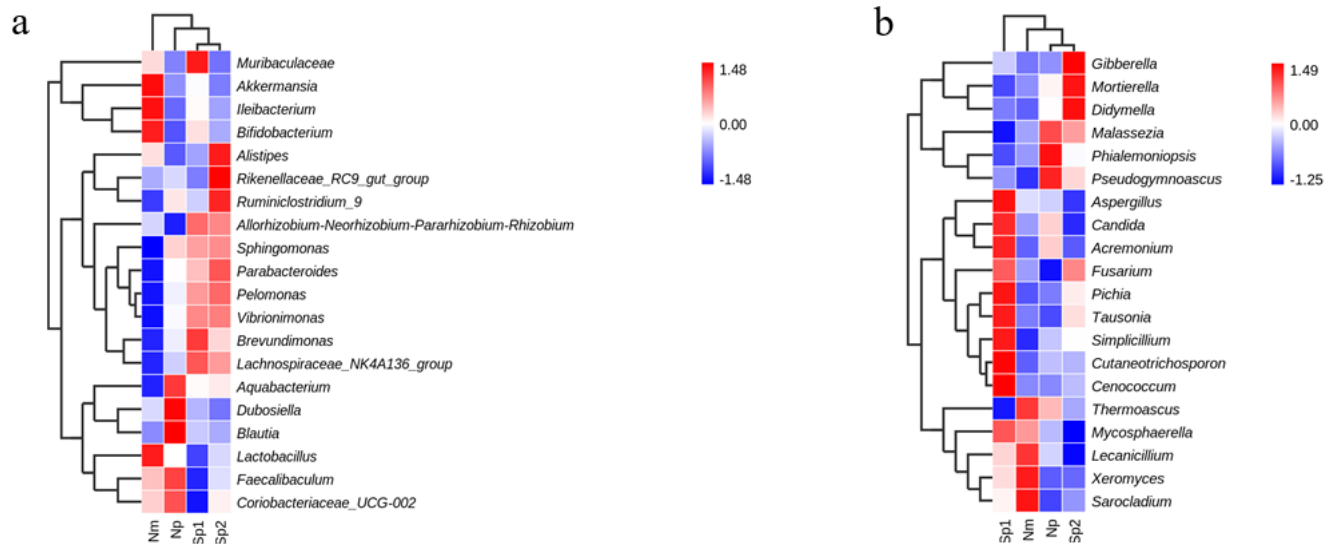


Figure 6

Heatmap analyses of abundant of bacteria (a) and fungi (b) species in each group at the genus level. The y axis is a neighbor-joining phylogenetic tree, each row is a different phylotype. The color of the spots in the right panel represents the mean relative abundance of the genus in each group. n = 3, in each group.

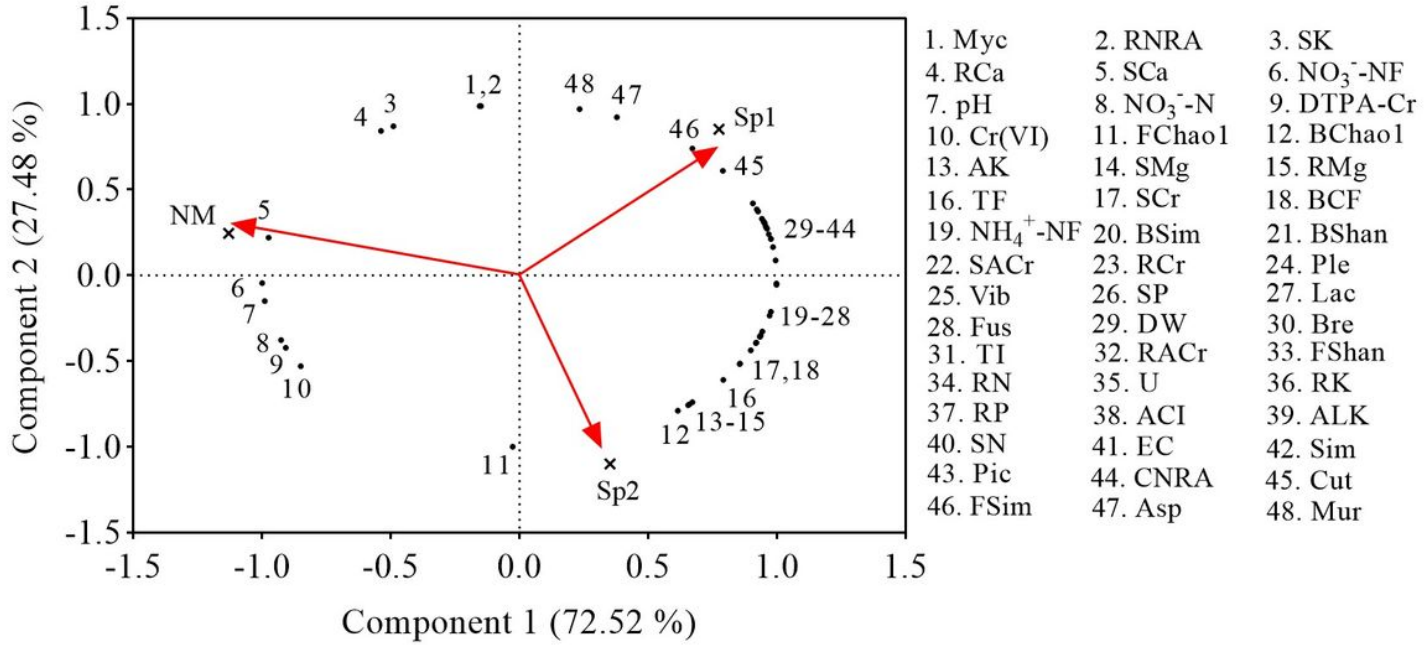


Figure 7

Principal component analysis (PCA) of soil properties (pH, NO_3^--N, DTPA-Cr, Cr(VI), available potassium (AK), Urease activity (U), NH_4^+-N, activity of Urease (U), acid phosphatase (Aci), alkaline phosphatase (Alk), Electrical conductivity (EC)), microbial index (Chao 1 of bacterial (BChao1) and fungi (FChao1), shannon of bacterial (BShan) and fungi (FShan), simpson of bacterial (BSim) and fungi (FSim), relative abundance of microbial (*Mycosphaerella* (Myc), *Pleomonas* (Ple), *Vibrionimonas* (Vib), *Lachnospiraceae* (Lac), *Fusarium* (Fus), *Brevundimonas* (Bre), *Simplicillium* (Sim), *Pichia* (Pic), *Cutaneotrichosporon* (Cut), *Aspergillus* (Asp) and *Muribaculaceae* (Mur)), and plant phenotype (Root nitrate reductase activity (RNRA), K concentration in shoot (SK), Ca concentration in root (RCa) and shoot (SCa), net NO_3^--N flux (NO_3^--NF), Mg concentration in shoot (SMg) and root (RMg), transportion factor (TF), Bioconcentration factor (BCF), Cr concentration in shoot (SCr) and root (RCr), net NH_4^+-N flux (NH_4^+-NF), tolerant index (TI), total accumulated Cr in shoot (SACr) and root (RACr), N concentration in root (RN) and shoot (SN), K concentration in root (RK) and shoot (SK), P concentration in root (RP) and shoot (SP), DW, coniferous nitrate reductase activity (CNRA)) exposed to Cr for 5 months.

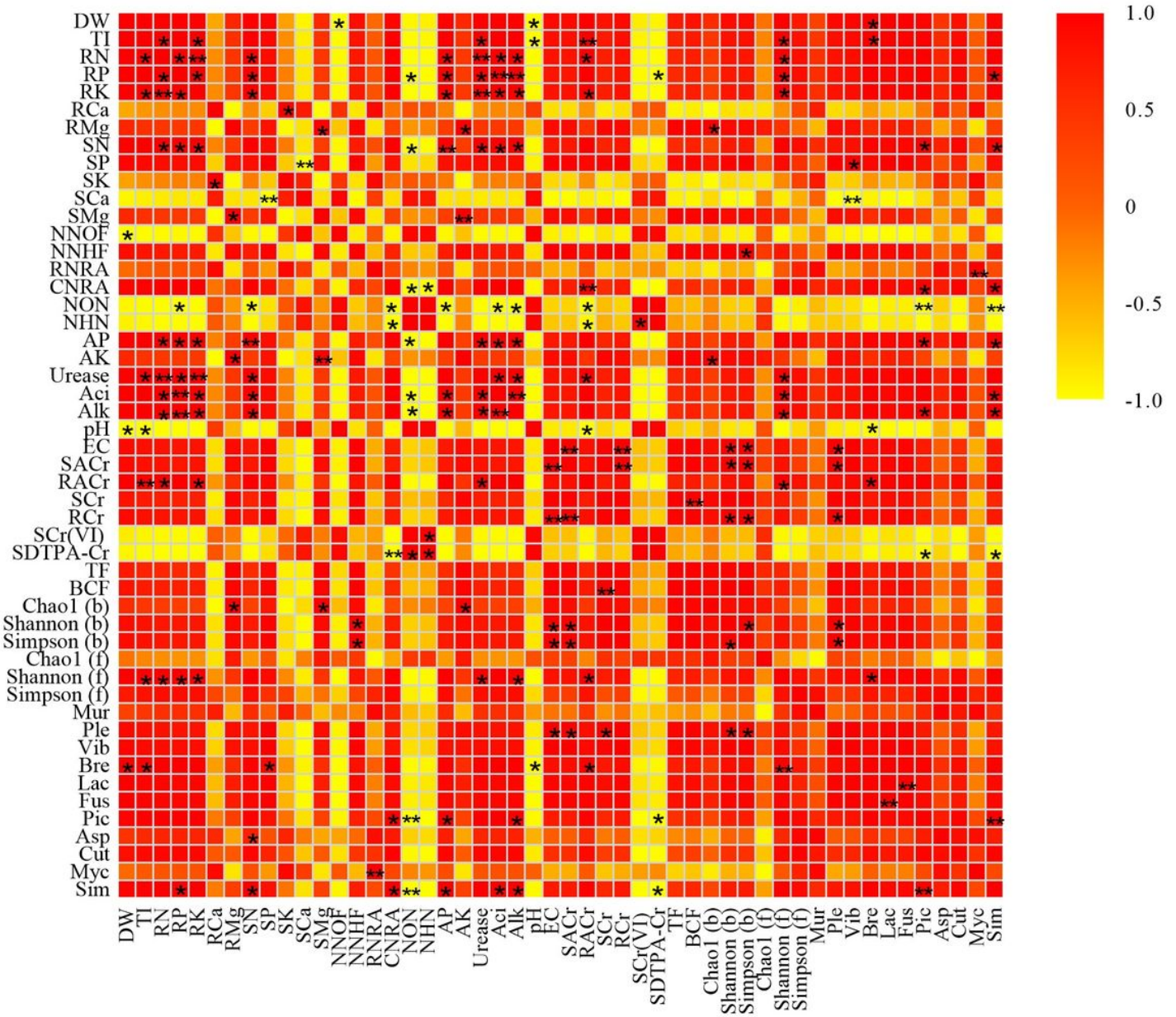


Figure 8

Pearson's correlation matrix of all plant, soil and microbiology properties. The abbreviated properties with their respective units are as follows - DW, dry weight (g); TI, Tolerant index; RN, Nitrogen concentration in root (mg/g); RP, Phosphorus concentration in root (mg/g); RK, Potassium concentration in root; RCa, Calcium concentration in root; RMg, Magnesium concentration in root; SN, Nitrogen concentration in shoot (mg/g); SP, Phosphorus concentration in shoot (mg/g); SK, Potassium concentration in shoot (mg/g); SCa: Calcium concentration in shoot; SMg, Magnesium concentration in shoot; NNOF, net NO_3^- flux (pmol/cm^2 s) on surface of root; NNHF, net NH_4^+ flux (pmol/cm^{2}s) on surface of root, RNRA, Nitrate reductase activity of root (μg $NO_2^-h^{-1}g^{-1}$FW); CNRA, Nitrate reductase activity of coniferous (μg $NO_2^-h^{-1}g^{-1}$FW); NON, NO_3^--N concentration in soil (mg/kg); NHN, NH_4^+-N concentration in soil (mg/kg); AP, available

phosphorus (mg/kg); AK, available potassium (mg/kg); Aci, acid phosphatase (U/g); Alk, alkaline phosphatase (U/g); SACr, Total accumulated Cr content in shoot (μg); RACr, Total accumulated Cr content in root (μg); SCr, Cr concentration in shoot (mg/kg), RCr, Cr concentration in root (mg/kg); SCr(VI), Cr(VI) concentration in soil (mg/kg); SDTPA-Cr, DTPA-Cr concentration in soil (mg/kg); TF, Transport factor; BCF, Bioconcentration factor; Chao1 (b), Chao1 index of bacterial; Shannon (b), Shannon index of bacterial; Simpson (b), Simpson index of bacterial; Chao1 (f), Chao1 index of fungi; Shannon (f), Shannon index of fungi; Simpson (f), Simpson index of fungi; Mur, *Muribaculaceae*; Ple, *Pleomonas*; Vib, *Vibrionimonas*; Bre, *Brevundimonas*; Lac, *Lachnospiraceae*; Fus, *Fusarium*; Pic, *Pichia*; Asp, *Aspergillus*; Cut, *Cutaneotrichosporon*; Myc, *Mycosphaerella*; Sim, *Simplicillium*. *: Significant correlation (p<0.05); **: Extremely significant correlation (p<0.01).

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