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RESEARCH ARTICLE

Impact of metal stress on the production of secondary metabolites in *Pteris vittata* L. and associated rhizosphere bacterial communities

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Abstract Plants adapt to metal stress by modifying their metabolism including the production of secondary metabolites in plant tissues. Such changes may impact the diversity and functions of plant associated microbial communities. Our study aimed to evaluate the influence of metals on the secondary metabolism of plants and the indirect impact on rhizosphere bacterial communities. We then compared the secondary metabolites of the hyperaccumulator *Pteris vittata* L. collected from a contaminated mining site to a non-contaminated site in Vietnam and identified the discriminant metabolites. Our data showed a significant increase in chlorogenic acid derivatives and A-type procyanidin in plant roots at the contaminated site. We hypothesized that the intensive production of these compounds could be part of the antioxidant defense mechanism in response to metals. In parallel, the structure and diversity of bulk soil and rhizosphere communities was studied using high-throughput sequencing. The results showed strong differences in bacterial composition, characterized by the dominance of Proteobacteria and Nitrospira in the contaminated bulk soil, and the enrichment of some potential human pathogens, i.e., *Acinetobacter*, *Mycobacterium*, and *Cupriavidus* in *P. vittata*'s rhizosphere at the mining site. Overall, metal pollution modified the production of *P. vittata* secondary metabolites and altered the diversity and structure of bacterial communities. Further investigations are needed to understand whether the plant recruits specific bacteria to adapt to metal stress.

Keywords Bacterial communities · Metal stress · *Pteris vittata* · Rhizosphere · Secondary metabolites

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Abbreviations

UHPLC-DAD-ESI/QTOF-MS	Ultrahigh-performance liquid chromatography with diode array detection coupled to electrospray ionization and quadrupole time-of-flight mass spectrometry
UV	Ultraviolet
ESI	Electrospray ionization
MSMS	Tandem mass spectrometry
ESI/MS^2	Electrospray ionization tandem mass spectrometry
HRMS	High-resolution mass spectrometry
^1H-NMR	Proton nuclear magnetic resonance
RT	Retention time

SPE	Solid phase extraction
PVP	Soil under *P. vittata* polluted
BSP	Bulk soil polluted
DLP	Soil under *Dicranopteris linearis* polluted
PVC	Soil under *P. vittata* control
HSD	Honest significant difference
PCA	Principal component analysis
ANOVA	Analysis of variance
CFU	Colony forming unit
QPCR	Quantitative real-time polymerase chain reaction
OTU	Operational taxonomic unit
DGGE	Denaturing gradient gel electrophoresis
DPPH	2,2-diphenyl-1-picrylhydrazyl

Introduction

The presence of heavy metals in the environment, which is largely due to anthropic activities in urban and industrial areas, represents a stress for all living organisms, impacting ecosystem structure and function, including soil microbial communities (Jaishankar et al. 2014; Singh et al. 2014). Although some metallic trace elements are essential for bacterial physiology (e.g., zinc (Zn), iron (Fe), copper (Cu)), others (e.g., cadmium (Cd), chrome (Cr), arsenic (As)) are toxic for bacterial cells and can alter diversity and function of bacterial communities depending on their concentrations in the environment (Epelde et al. 2012; Lenart-Boroń and Boroń 2014). Metal pollution then represents a potential risk for bacterial community diversity and functions and then soil functioning (Azarbad et al. 2013; Singh et al. 2014).

Plants adapt to heavy metal stress by modifying their metabolism including the production of secondary metabolites in plant tissues, although less studied than primary ones (Singh et al. 2016). On the other hand, plants are known to be major drivers of soil microbial community structure and functioning through root exudation (Hartmann et al. 2008; Michalet et al. 2013). Changes in metabolism might then impact diversity and functions of microbial communities associated to leave (i.e., phyllosphere) or root (i.e., rhizosphere) systems. A recent theory named "Plants call for support," hypothesizes that pollution-induced changes in root exudation might select for microbial communities bearing beneficial traits for plants (e.g., degradative capacities), allowing a better adaptation under contaminant stress (Thijs et al. 2016).

In Vietnam, heavy metal pollution in soils resulting from anthropization, including mining activities, has been increasing in the last few decades. Recent study summarized that about 5000 mines of ore have been explored, and the area of abandoned mines was more than 3700 ha (Anh et al. 2011). In recent years, many researches have been focused on phytoremediation by hyperaccumulators in order to remove heavy metals from mining sites in Vietnam (Nguyen et al. 2011; Anh et al. 2011). However, it has not yet been evaluated how metal contamination alters the secondary metabolism of these plants as well as their effect on associated rhizosphere communities. Here we have used *Pteris vittata* L. as a plant model since this indigenous fern has been reported for its great potential to uptake As and to tolerate high Zn and Pb level in Ha Thuong mining site (Anh et al. 2011).

Pteris vittata L., commonly known as Chinese brake fern or Ladder brake fern, is a hyperaccumulator belonging to the Pteridaceae family and is found in tropical and sub-tropical regions like South East Asia, Africa, or Australia. This plant was reported as the first known As hyperaccumulator (Ma et al. 2001). Arsenic is essentially accumulated in its fronds at very high levels (up to 22,630 mg/kg), but the concentration of this metal in the roots is also non-negligible (100 mg/kg) (Ma et al. 2001; Wang et al. 2002). *P. vittata* has been extensively studied for the phytoremediation of As-contaminated soils, due to its strong As accumulation as well as its capacity to take up many forms of As (Ma et al. 2001; Tu and Ma 2002; Tu et al. 2002; Danh et al. 2014). *P. vittata* is also able to accumulate other metals such as Zn (up to 737 mg/kg in leaves) (An et al. 2006), and its tolerance to high contamination level of Pb, Zn, and Cu was also reported (Kachenko et al. 2007; Anh et al. 2011). Besides, this plant has been explored for its pharmacological properties like hypoglycemic (Paul et al. 2012), anti-inflammatory, platelet aggregation, and antitumor activities (Gong et al. 2007). Some *P. vittata* secondary metabolites were also examined for their antimicrobial properties (rutin) (Singh et al. 2008) and their impacts on visual process (rutin, flavones glycosides…) (Wahid et al. 2016). To our knowledge, although several studies have investigated the phytochemical composition of *P. vittata* extracts and the impacts of *P. vittata* on heavy metal content in soils (Tu et al. 2002; Nguyen et al. 2011; Gracelin et al. 2012; Jaishee and Chakraborty 2015; Wahid et al. 2016), the effect of these pollutants on its metabolism remains unknown.

Thus, our objectives were (i) to compare the secondary metabolite profiles of each part of *P. vittata* grown on contrasted contaminated areas, in order to evaluate the effect of metal pollution on secondary metabolism of plants colonizing metalliferous sites, and (ii) to evaluate the indirect influence of these changes in metabolism on the rhizosphere bacterial community composition. Metabolic profiling of roots, stems, and leaves of *P. vittata* was based

on UV-absorbing compounds, thus targeting more specifically phenolics and other aromatic compounds. Changes in bacterial community structure were assessed using a metataxogenomic analysis approach.

Materials and methods

Site, plant, and soil sampling

The studied site is located at Ha Thuong Commune, Dai Tu district, Thai Nguyen Province, Vietnam (Fig. 1). At that site, Sn-ore mining activities have been operated from 1988 to 2007. These operations led the contamination with heavy metals including As, Pb, Zn, Cu, and Cd in soils. In 2007, in the Vietnam National Research Projet, No. KC08.04/06-10, for phytoremediation of mining areas in Thai Nguyen, *P. vittata*, *Pityrogramma calomelanos*, and *Dicranopteris linearis* were cultured to remove As and Cd from soil. However, the concentration of Cu and Pb in soil still exceeds the allowable limit according to "National technical regulation on the allowable limits of heavy metals in the soils" (Table 1).

Ten mature *P. vittata* individuals were collected in Ha Thuong mining site and five individuals were sampled at the same growth stage in a non-contaminated site (Academy of Science and Technology, in Hoang Quoc Viet Street, Cau Giay district, Hanoi, Vietnam). All samples were identified by Prof. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology. Voucher specimens were deposited in the Department of Bioactive Products, Institute of Marine Biochemistry, Vietnam Academy of Science and Technology.

From the mining site, rhizosphere soil samples were collected under *P. vittata* (PVP) and *D. linearis* (DLP) (another fern cultured in this site and used in this study as a comparative polluted rhizosphere soil). Nearby, bulk soil samples (BSP) (i.e., soil with no vegetal cover) were collected. Rhizosphere soil samples were also collected under *P. vittata* at the Vietnam Academy of Science and Technology and will be further considered as a control (PVC) (non-polluted soil). All soils were collected from the upper layer (0–15 cm) and stored at ambient temperature for no longer than 2 weeks before use. Soil characteristics are shown in Table 1 and were measured by the Laboratory of Soil Analysis (INRA Arras, France) using standard methods. Metal content in soil was analyzed by inductively coupled plasma mass spectrometry (ICP-MS) at the Centre des Recherches Pétrographiques et Géochimiques (CRPG) of Vandoeuvre-les-Nancy (France) and metal content in plant parts was analyzed by the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology.

Sample preparation for the analysis of plant metabolites

Each plant was carefully excavated and dried, and each part (roots, stems, and leaves) was separated before being crushed into fine powder using mortar and liquid nitrogen. Plant metabolites were extracted by immersion of 5 g of dried samples in 100 ml of absolute MeOH in an Erlenmeyer, followed by ultra-sonication for 15 min at room temperature and then filtration. Extraction process was repeated three times. The extracts were pooled and evaporated under reduced pressure, stored at −20 °C, and then dissolved in absolute MeOH to a concentration of 10 mg/mL before analysis.

For UHPLC-DAD-ESI/QTOF analysis, stem and leave extracts were prepared in the same conditions described above while root extracts were dissolved in MilliQ water and further divided into fractions by using solid phase extraction (SPE) on Bond Elut® C18 SPE cartridges (Agilent Technologies®, Santa Clara, CA, USA). Elution was performed with a gradient of H_2O/MeOH (100:0; 80:20; 0:100). The fraction H_2O/MeOH (80:20) was evaporated then dissolved in MeOH absolute with a final concentration of 10 mg/mL before analysis.

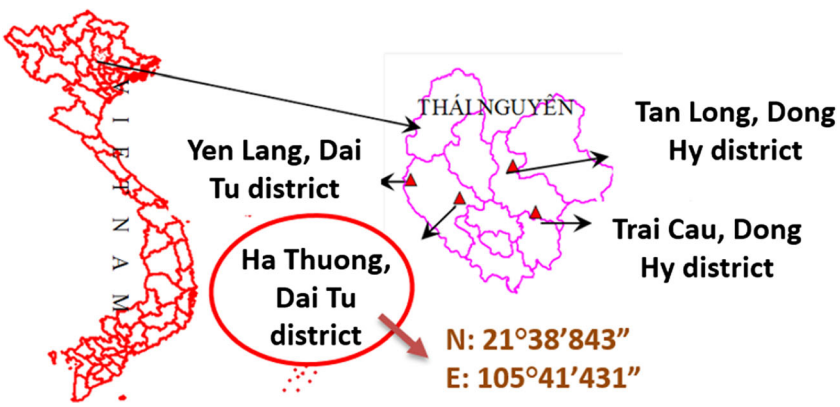

Fig. 1 Location of the Sn-ore mining site at Ha Thuong, Dai Tu District, Thai Nguyen Province (Vietnam)

Table 1 Physical and chemical characteristics of the rhizosphere and bulk soil

Properties	Unit	PVP	BSP	DLP	PVC	Limit industrial soil[b]
Clay (<2 μm)	g/kg	321	119.5	186	150	–
Silt	g/kg	537	163	356.5	528	–
Sand	g/kg	142	717.5	457.5	322	–
Organic matter	g/kg	53.4	1.22	3.025	19.1	–
Organic carbon (C)	g/kg	30.9	0.7065	1.745	11.05	–
Nitrogen (N) total	g/kg	2.385	0.0705	0.1895	1.06	–
C/N	–	13	10.165	8.995	10.4	–
pH	–	5.33	2.45	4.19	8.17	–
CEC[a] cobaltihexamine	cmol + /kg	13.2	2.175	7.57	11.05	–
Copper (Cu)	mg/kg	436	3150	82.45	115	300
Lead (Pb)	mg/kg	279.5	600	106.7	132.5	300
Arsenic (As)	mg/kg	2.78	nd	nd	2.6	25

BSP, bulk soil at the mining site; PVP and DLP, rhizosphere soils of *Pteris vittata* and *Dicranopteris linearis*, respectively, at the mining site, and PVC, rhizosphere soil of *P.vittata* at the control site)

[a] Cation-exchange capacity

[b] According to Vietnam National Technical Regulation on the allowable limits of heavy metals in the soils (QCVN 03-MT 2015/BTNMT)

UHPLC-DAD-ESI/QTOF analysis

UHPLC-DAD-ESI/QTOF analysis was performed on an Agilent Infinity® 1290 system (Agilent Technologies®) coupled to a UV/vis DAD detector and equipped with a QTOF 6530 (Agilent Technologies®) detector controlled by MassHunter® software (Agilent Technologies®). Analyte separation was carried-out on a Poroshell® 120 EC-C18 column (100 mm × 3.0 mm, 2.7 μm). The following gradient was run using 0.4% aqueous formic acid (solvent A) and acetonitrile (solvent B): 0 min, 1% B; 1.5 min, 1% B; 12 min, 30% B; 18 min, 100% B; 19 min, 100% B. The flow rate and column temperature were 1.2 mL/min and 50 °C, respectively, and 2.0 μL of sample extracts was injected. The signals were detected at four wavelength λ 254, 280, 320, and 360 nm and UV spectra recorded between 190 and 600 nm. The chromatograms recorded at λ 280 nm were selected for statistical analysis.

The ESI source was optimized as follows: positive and negative ionization mode in auto-MSMS, scan spectra from m/z 100 to 3000, capillary voltage 2.5 kV, nozzle voltage 2 kV, fragmentor 120 V, and fixed collision-induced dissociation (CID) energy at 20 eV. Nitrogen was used as the nebulizing gas with a flow rate of 11 L/min and a temperature of 310 °C at 40 psi.

Metabolites annotation

The annotation of metabolites in *P. vittata* extracts was based on UV, HRMS, MSMS spectra, and relative RTs. Data were compared with previous literature report for compounds in *Pteris* spp. or in other genus.

Statistical analysis

To assess differences in metabolite composition between plants growing in non-polluted and in polluted area, RTs of peaks in chromatograms at wavelength λ 280 nm were aligned and their relative intensities integrated in a matrix for each plant part to perform the principal component analysis (PCA) using R® software, version 3.1.2 (R Core Team 2014). The compounds responsible for the discrimination between polluted and non-polluted samples were further analyzed using ANOVA followed by Tukey's honest significant difference (HSD) tests after data were controlled for normality and homoscedasticity. Those analyses were conducted at $p < 0.05$.

Soil DNA extraction and metagenomic analysis

Total DNA was extracted from 0.5 g of each bulk and rhizosphere soil sample with three replicates using a FastDNA® SPIN Kit for Soil (MP Biomedicals, Solon, OH, USA) and then purified on S-400-HR mini-columns (Pharmacia, St Quentin Yvelines, France) following the manufacturer's instructions. The DNA extracts were resolved by electrophoresis in 0.8% agarose gels, stained with ethidium bromide, and photographed using a Gel Doc 1000 camera (Bio-Rad, Ivry sur Seine, France). DNA concentration was estimated using a NanoDrop® ND-1000 spectrophotometer (Labtech International, Paris, France)

at a 260 nm wavelength. Triplicate DNA extractions were performed from all samples. Replicates were then pooled for further metagenomic analysis.

16S rRNA genes of pooled DNA samples were sequenced at the DTAMB/ Biofidal platform (University of Lyon 1, France) using an Illumina's Miseq platform and paired end reads. In total, 1,254,982*2 valid sequences for bacteria were obtained from four samples. Metagenomic analysis was carried-out following the Mothur MiSeq standard operating procedure (SOP) pipeline v1.35.1 (http://www.mothur.org/wiki/MiSeq_SOP) developed by the Schloss lab (Department of Microbiology & Immunology, University of Michigan, USA) (Schloss et al. 2009; Kozich et al. 2013). Briefly, the contigs of paired end reads 1 and 2 were constructed using the *make.contigs* command, and 1,245,982 sequences with an average length of 457 bp were produced. After removing the sequences with ambiguous bases, and those longer than 550 bp or shorter than 400 bp with the *screen.seqs* command, a total of 338,298 high-quality sequences were obtained with an average length of 448 bp per sequence. The only unique sequences were obtained using *unique.seqs* command and the *align.seqs* command were then operated to align the data with mothur's SILVA bacterial 16S reference alignment v123 (https://www.arb-silva.de/) (Quast et al. 2013). After screening the unique sequences, the remaining reads were then pre-clustered to denoise sequences within each sample and chimeric sequences were removed using *chimeric.uchime* command (http://drive5.com/uchime) (Edgar et al. 2011). Finally, a range from 24,458 to 66,753 sequences with an average number of 38,566 sequences per sample were obtained and then subsampling to 24,458 sequences. Each sequence was then classified against the RDP 16S rRNA training set v9 using a naïve Bayesian classifier, at an 80% confidence level (Wang et al. 2007). The sequences were regrouped into operational taxonomic units (OTUs) with a 3% divergence threshold using *cluster.split* command (http://www.mothur.org/wiki/Cluster.split). The Chao1 richness estimator and the Np Shannon diversity were also calculated.

Bacterial quantification using culture enumeration and qPCR

Microorganisms were extracted by blending 5 g of soil samples with 50 mL of a 0.8% (*w*/*v*) sterile NaCl solution for 90 s in a Waring blender (Eberbach Corporation, Michigan, USA). The homogeneous soil suspension was serially diluted tenfold in sterile saline solution and 100 μL of appropriate dilutions were spread. The viable heterotrophic microflora was enumerated on tenfold diluted tryptic soy agar medium (TSA1/10) (Oxoïd, Dardilly, France) supplemented with cycloheximide (200 mg/L) to impair the growth of fungi. Three plates were inoculated per dilution and bacterial colonies were counted after 5 days of incubation at 28 °C.

Real-time PCR amplification (qPCR) was performed using a LightCycler 480 system (Roche Diagnostics, Meylan, France) with the LightCycler® 480 software, version LCS480 1.5.0.39 (Roche Diagnostics). The primers 5′-TCCATGAAGTCGGAATCGCTAG-3′ and 5′-CACTCCCATGGTGTGACGG-3′ were used (Invitrogen, Cergy Pontoise, France). The mix reactions were performed with the SsoFast™ EvaGreen® Supermix (Bio-Rad) as specified by the manufacturer. Five microliters of template DNA (corresponding to 0.2 and 1 ng of DNA) was added, and deionized water was used to reach a final volume of 25 μL. Negative controls without template DNA were run in triplicate. Each reaction was run in duplicate with both DNA template concentrations using the following cycle conditions: 1 cycle at 95 °C for 10 min followed by 40 cycles of 95 °C for 10 s and 60 °C for 10 s.

Results

Metabolic profiling of UV-absorbing compounds in *P. vittata* extracts

UV-based metabolite profiling was carried out on root, stem, and leave extracts of fifteen *P. vittata* samples, ten of which were collected from contaminated soil, in order to compare the metabolite profiles of plants from metal-polluted and non-polluted areas. Chromatograms at λ 280 nm showed strong differences between samples from these two areas as shown by the comparison of typical UHPLC/DAD chromatograms (Figs. S1, S2, S3**)**. Concentrations of compounds (i.e., absolute areas) were also affected but in order to compare the variations in global extract compositions, relative area was chosen (which also enabled to attenuate the variability in total extract concentrations). This suggests that there are qualitative and quantitative changes in the secondary metabolism of plants from diversely contaminated soils.

PCA allowed to highlight differences between the metabolic profiles of plant grown on contaminated soils from those grown in non-contaminated area, and this was especially the case for stems and leaves (Fig. 2). Concerning roots, the discrimination was less pronounced (Fig. 2a), probably because of the variability observed in compound relative intensities, which might be due to the extra SPE separation procedure realized for these extracts. In leaves and stems (Fig. 2b, c), the discrimination was better and each group could be well separated along axis 1.

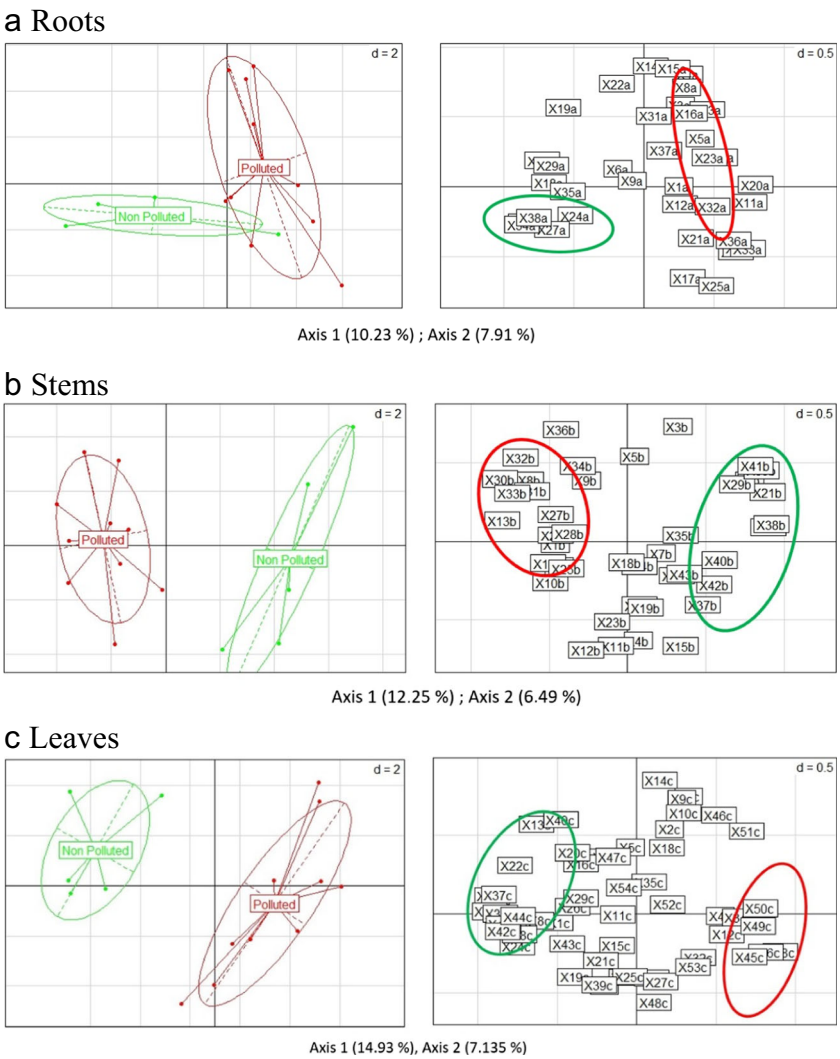

Fig. 2 PCA performed on UV chromatographic profiles obtained for each extract of *P. vittata*: roots (**a**), stems (**b**), and leaves (**c**) grown on non-polluted and polluted soil. *Each point on the left plots* represents one extract and the *circles* indicate the type of soil on which plants grew. *Each point on the right plots* represents one peak and the *circles* indicates the discriminant compounds

Secondary metabolites affected by metal contamination

Based on PCA, the compounds involved in the different group segregation were highlighted (Fig. 2). In order to identify the discriminating peaks in different conditions, ANOVA followed by Tukey's HSD test was carried-out for these compounds and only those that showed a statistically significant difference and representing at least 1% of the total area in one condition are highlighted. In root extracts, six discriminant compounds were characterized, two of which were found significantly in higher proportions in the polluted condition compared to the non-polluted one (**8a**, **32a)**, while four were found in lower proportions (**26a**, **28a**, **34a**, **38a**) (Fig. 3a, b). Regarding stems, five compounds were identified with significantly increased proportions in the case of polluted soil (**13b**, **20b**, **30b**, **32b**, **33b**), while three were found with decreased proportions (**24b**, **38b**, **39b**) (Fig. 3c, d). Concerning leaves, six peaks were found as discriminating, of which four were in higher proportions with contamination (**28c**, **36c**, **45c**, **49c**) and two presenting lower proportions (**33c**, **37c**) (Fig. 3e, f).

Annotation of discriminant compounds

The discriminant compounds are listed in Tables 2 and 3 with their identification based on relative RTs, UV, and mass spectra. In roots, most of the peaks involved in the segregation of different groups were identified as hydroxycinnamic acid derivatives. Considering stems and leaves, flavonol glycoside derivatives seemed to be the major discriminant compounds.

Annotation of discriminant compounds in root extracts

Discriminant compounds annotated in root extracts were distributed in two major classes: hydroxycinnamic derivatives (**8a**, **26a**, **28a**, **34a**, **38a**) and procyanidin (**32a**), according to their respective specific UV spectra (i.e., a strong absorption band at 310–320 nm with a shoulder at 300 nm and a weak absorption band at 276–278 nm, respectively). The most abundant compound, **32a**, was detected at RT 8.328 min and found in higher proportion in roots of plants growing in metal-polluted area. Its mass spectra showed a pseudo-molecular ion

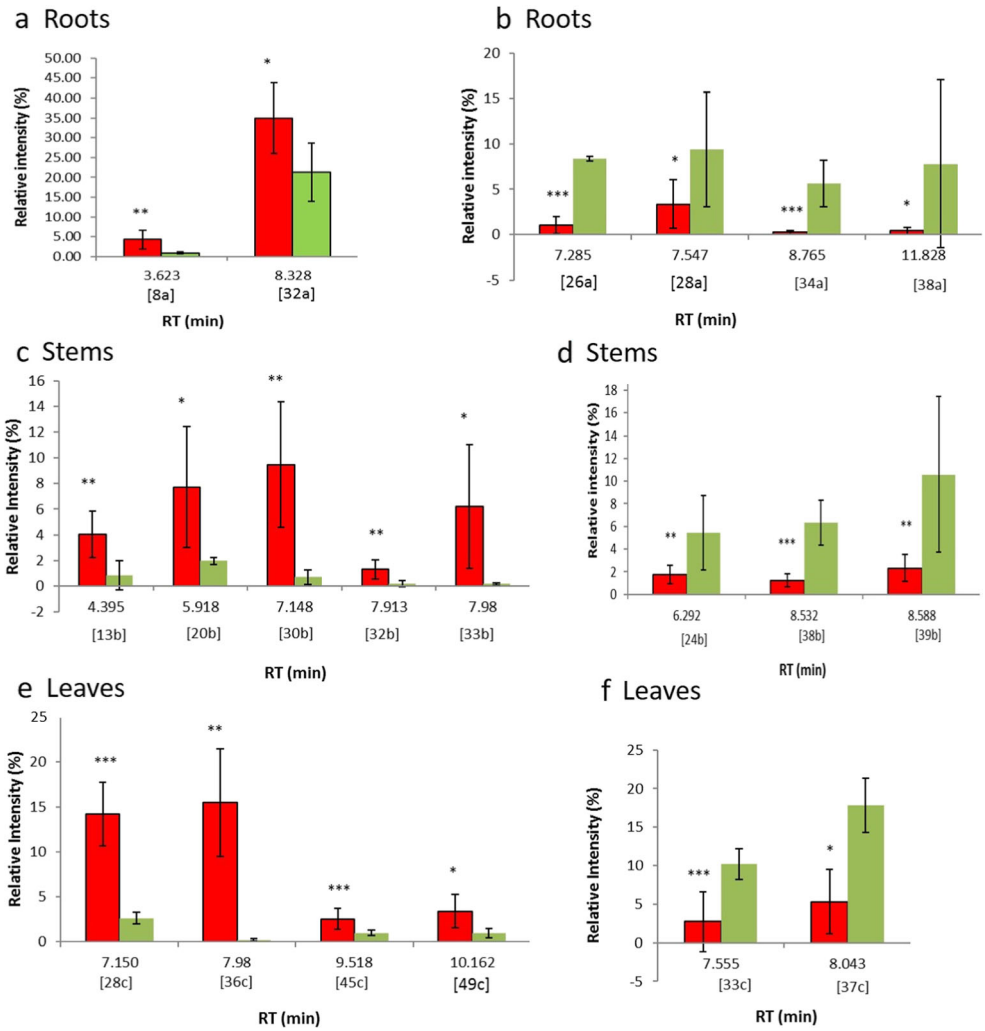

Fig. 3 *P. vittata* root, stem, and leave secondary metabolites affected by the type of soil. Relative intensities of discriminant chromatographic peaks: increasing compounds (**a**, **c**, **e**) and decreasing compounds (**b**, **d**, **f**) in *P. vittata* roots, stems, and leaves, respectively. *Green bars* indicate extract obtained from non-polluted soils and *red bars* from polluted ones. Statistical differences between type of soil are indicated using characters *, **, *** (Tukey's HSD test, significativity codes '***' $p < 0.001$, '**' $p < 0.01$, '*' $p < 0.05$). Compound numbers are indicated within brackets below Rts.

at m/z 863.1826 ($[M-H]^-$) and m/z 865.2013 ($[M+H]^+$) corresponding to the molecular mass of 864 Da with the formula $C_{45}H_{36}O_{18}$ suggesting a procyanidin trimer. Moreover, the ESI−/MS^2 spectra of this compound produced the fragmentation ions at m/z 773 and m/z 289, indicative of the presence of an A-type inter-flavonoid linkage. Based on the literature data (Gu et al. 2003; Qiang et al. 2015), this compound was tentatively identified as A-type procyanidin trimer, with the (epi)catechin-A-(epi)catechin-(epi)catechin connection.

Among the hydroxycinnamic derivatives, one compound (**8a**) was found to be increased and four compounds (**26a**, **28a**, **34a**, **38a**) were found to be decreased in metal-polluted area. For compound **8a** (RT 3.623 min), the ($[M-H]^-$) ion at m/z 353.0878 and the ($[M+H]^+$) ion at m/z 355.1041 suggested the molecular weight of 354 Da corresponding to the formula $C_{16}H_{18}O_9$. ESI^+/MS^2 spectra of this compound produced a base peak at m/z 163.0394 ($[M+H\text{-}C_7H_{11}O_6]^+$), corresponding to the loss of a quinic subunit. Moreover, ESI^-/MS^2 data generated a base peak at m/z 191.0533 accounting for the loss of a caffeic acid subunit ($[M\text{-}H\text{-}C_9H_6O_3]^-$). Considering the relative intensity of two secondary ions (m/z 179 and m/z 135) in ESI^-/MS^2 spectra, compound **8a** was identified as 3-*O*-caffeoylquinic acid (chlorogenic acid) according to published data (Clifford et al. 2003). Using the same reasoning, compound **28a** was tentatively identified as 4-*O*-*p*-coumaroylquinic acid.

Among the other hydroxycinnamic acids, compound **26a** and **38a**, detected at RT 7.285 and 11.828 min respectively, showed the same UV characteristic and ESI^+/MS data as compound **8a** (Table 2), but with a molecular formula of $C_{18}H_{20}O_{10}$ (M = 396), indicative of an acetylation. ESI^-/MS^2 allowed to identify both a major product ion at m/z 233, corresponding to the acetylated quinic acid moiety ($C_9H_{14}O_7$ = 234), and a major ESI^+/MS^2 product ion at m/z 163, accounting for the caffeic acid moiety. To our knowledge, the 3-*O*-acetyl-4-*O*-caffeoylquinic acid and the 3-*O*-caffeoyl-5-*O*-acetylquinic acid were identified using ^{1}H-NMR and ESI/MS data (Whitaker and Stommel 2003), but no literature allow to differentiate these isomers

Table 2 Annotation of the discriminating compounds in *Pteris vittata* root extracts

Cpd number	RT (min)	UV (nm)[a]	m/z $[M-H]^-$	m/z MS^2 (intensity, %)	m/z $[M+H]^+$	m/z MS^2 (intensity, %)	Formula	Putative identification	References
8a	3.623	244, 296sh, 322	353.0878	191 (100), 179 (64), 135 (34)	355.1041	163 (100)	$C_{16}H_{18}O_9$	3-caffeoylquinic acid	(Clifford et al. 2003)
26a	7.285	295sh, 322	395.0984	233 (100), 179 (22), 173 20), 135 (13),	397.0392	163 (100)	$C_{18}H_{20}O_{10}$	Acetylated caffeoylquinic acid	(Whitaker and Stommel 2003)
28a	7.547	294sh, 310	337.0924	173 (100), 163 (19)	339.1060	147 (100), 119 (6)	$C_{16}H_{18}O_8$	4-*O*-*p*-coumaroylquinic acid	(Clifford et al. 2003)
32a	8.337	226, 278	863.1826	863 (100)), 711 (56), 573 (27), 289 (23)	865.2013	865 (100), 713 (61), 533 (87), 287 (36)	$C_{45}H_{36}O_{18}$	A-type procyanidin trimer	(Gu et al. 2003; Qiang et al. 2015)
34a	8.765	254sh, 296sh, 314	379.1025	233 (64), 163 (100), 119 (90)	381.1198	147 (100)	$C_{18}H_{20}O_9$	Acetylated coumaroylquinic acid	
38a	11.833	244, 296sh, 326	395.0974	233 (100), 179 (15), 173 (26), 135 (11)	397.1147	163 (100)	$C_{18}H_{20}O_{10}$	Acetylated caffeoylquinic acid	(Whitaker and Stommel 2003)

sh spectral shoulder, *Cpd* compound

[a] Maximum spectral absorbance wavelengths (nm)

Table 3 Annotation of the discriminating compounds in *Pteris vittata* stem and leave extracts

Cpd number	RTs (min)	UV (nm)[a]	m/z $[M-H]^-$	m/z MS^2 (intensity, %)	m/z $[M + H]^+$	m/z MS^2 (intensity, %)	Formula	Putative identification	References
13b	4.395	260, 286,	nd	nd	nd	nd	nd		
20b	5.918	222, 268, 296sh, 326	593.1530	593 (100), 473 (11)	595.1688	595(62), 577 (70), 457 (100)	$C_{27}H_{30}O_{15}$	Di-C-glycosylated Flavone	
24b	6.292	286sh, 320	nd	nd	nd	nd	nd_3		
30b and 28c	7.148	256, 266sh, 300sh, 352	609.1461	609 (100), 463 (0.4), 301 (13)	611.1636	465 (10), 303 (100),	$C_{27}H_{30}O_{16}$	Quercetin-3-*O*-rutinoside	(Vukics and Guttman 2010; Crupi et al. 2014)
33c	7.555	254, 296sh, 336	933.2289	933 (100), 711 (27), 609 (1), 463 (0.2), 301 (7)	935.2477	471 (42), 465 (53), 325 (100), 303 (14), 163 (59)	$C_{42}H_{46}O_{24}$	Quercetin-3-*O*-(caffeoyl)rutinoside-7-*O*-hexoside	(Lin and Harnly 2010)
32b	7.913	264, 300sh, 344	593.1525	593 (100), 285 (33)	595.1684	449 (7), 287 (100)	$C_{27}H_{30}O_{15}$	Kaempferol-3-*O*-rutinoside	(Crupi et al. 2014)
33b and 36c	7.98	264, 300sh, 344	461.0711	285 (100)	463.0892	287 (100)	$C_{21}H_{18}O_{12}$	Kaempferol-3-*O*-glucuronide	(Kajdžanoska et al. 2010)
37c	8.043	244, 266, 295sh, 332	917.2345	917 (100), 755 (75), 593 (3), 447 (0.7), 285 (20)	919.2354	773 (15), 471 (37), 449 (53), 325 (100), 287(18), 163 (69)	$C_{42}H_{46}O_{23}$	Kaempferol-3-*O*-(caffeoyl)rutinoside-7-*O*-hexoside	(Lin and Harnly 2010)
38b	8.532	276, 310sh	nd	nd	nd	nd	nd		
39b	8.588	282, 316sh	nd	nd	nd	nd	nd		
45c	9.518	248, 266sh, 298sh, 334	771.1770	771 (100), 609 (6), 469 (0.2), 301 (40)	773.1944	471 (36), 325 (100), 303 (69), 163 (97)	$C_{36}H_{36}O_{19}$	Quercetin-3-*O*-(caffeoyl)rutinoside	(Martucci et al. 2014)
49c	10.162	246sh, 266, 295sh, 330	755.1812	755 (100), 593 (2), 469 (7), 285 (88)	757.1989	471 (30), 325 (100), 287 (66), 163(89)	$C_{36}H_{36}O_{18}$	Kaempferol-3-*O*-(caffeoyl)rutinoside	(Martucci et al. 2014)

sh spectral shoulder, *Cpd* compound, *nd* not detected

[a] Maximum spectral absorbance wavelengths (nm)

based on ESI/MS^n spectra. Thus, these two compounds could be annotated as acetylated caffeoylquinic acid isomers.

Using the same reasoning, compound **34a** was annotated as acetylated coumaroylquinic acid.

Annotation of discriminant compounds in stem and leave extracts

The most discriminant and abundant compounds detected in *P. vittata* stem and leave extracts were identified as flavonol glycosides derivatives, a major class of plant secondary metabolites which have already been well described in *P. vittata* (cf cited literature in Table 3). Four quercetin derivatives (peak **30b** in stems and **28c**, **33c, 45c** in leaves) as well as five kaempferol derivatives (peak **32b**, **33b** in stems and **36c**, **37c**, **49c** in leaves) were identified according to their typical MS^n fragmentations (providing fragmentation ions at m/z 303 and m/z 287 in positive mode corresponding to respective aglycones) together with their specific UV spectra (i.e., absorption band II lower than 260 nm for quercetin derivatives and higher than this value for kaempferol derivatives; (Mabry et al. 1970)). Among these compounds, two were found in higher proportion in both stems and leaves: **30b** and **28c** (RT 7.150 min); **33b** and **36c** (RT 7.980 min).

Compound **30b**/**28c** possess UV characteristic of 3-*O*-substituted quercetin (Mabry et al. 1970) and showed pseudomolecular ions at m/z 609.1416 ($[M-H]^-$) and at m/z 611.1636 ($[M+H]^+$) corresponding to the molecular formula $C_{27}H_{30}O_{16}$. The ESI/MS^2 spectra show major ion at m/z 303 in positive mode and m/z 301 in negative mode, accounting for a loss of 308 Da corresponding to the loss of a deoxyhexose and a hexose unit. The comparison with literature suggested that the disaccharide moiety is a rutinoside type (Vukics and Guttman 2010), thus this compound was putatively identified as quercetin-3-*O*-rutinoside (= rutin) (Crupi et al. 2014).

Compound **45c** (RT 9.518) showed pseudomolecular ions at m/z 773.1944 ($[M+H]^+$) and at m/z 771.1770 ($[M-H]^-$) allowing to establish the molecular formula of $C_{36}H_{36}O_{19}$. Its ESI^-/MS^2 spectra showed product ions at m/z 609 (indicative of the loss of a caffeoyl unit), m/z 609 at very low intensity and m/z 301 (indicating the loss of 308 Da corresponding to a disaccharide of type rutinoside). The ESI^+/MS^2 product ion at m/z 303 and ESI^-/MS^2 product ion at m/z 301 reveal the presence of a quercetin aglycone. In addition, the fragment ions at m/z 325 and m/z 163 correspond to a caffeoyl-hexose unit and a caffeoyl unit, respectively. Compound **45c** was thus annotated as quercetin-3-*O*-(caffeoyl)rutinoside. Using the same reasoning and by comparison with literature, compound **49c** was tentatively identified as kaempferol-3-*O*-(caffeoyl)rutinoside (Martucci et al. 2014).

Compound **33c** (RT 7.555), the only quercetin derivatives identified in lower proportions in leaves with the influence of metal, showed pseudomolecular ions at m/z 935.2477 $[M+H]^+$ and at m/z 933.2289 $[M-H]^-$ with a molecular formula of $C_{42}H_{46}O_{24}$. The ESI^-/MS^2 spectra showed product ions at m/z 771 (corresponding to the loss of a hexose unit), m/z 609 (loss of a caffeoyl unit), m/z 463 (indicative of the loss of a rhamnosyl unit), m/z 303 (loss of a hexosyl unit). This led to a putative identification of this compound as quercetin-3-*O*-(caffeoyl) rutinoside-7-*O*-hexoside. Using the same reasoning and by comparison with literature concerning its characteristic UV spectrum (Lin and Harnly 2010), compound **37c** was tentatively identified as kaepferol-3-*O*-(caffeoyl) rutinoside-7-*O*-hexoside.

Compound **33b**/**36c** detected at RT 7.98 min showed pseudomolecular ions at m/z 461.0718 ($[M-H]^-$) and at m/z 463.0893 ($[M+H]^+$), corresponding to the molecular formula of $C_{21}H_{18}O_{12}$. The ESI^-/MS^2 spectra showed a base peak at m/z 285 corresponding to the kaempferol aglycone and to the loss of a glucuronide moiety (176 Da). Therefore, following literature, compound **33b**/**36c** was tentatively identified as kaempferol-3-*O*-glucuronide (Kajdžanoska et al. 2010).

Although the majority of abundant and discriminant compounds in *P. vittata* stems and leaves were putatively annotated, minor UV-absorbing compounds still remain unidentified, in part due to their difficulty to be ionized.

Bacterial abundance and community structure

Total heterotrophic microflora varied between 7.01 (±5.06) × 10^3 colonies forming unit (CFU)/gram in the bulk soil of the mining site (sample BSP) and 46.6 (±13.9) × 10^6 CFU/g in the rhizosphere of *P. vittata* at the mining site (sample PVP) (Table 4). Based on qPCR quantification, the number of 16S rDNA copies varied from 0.557 (±0.0680) × 10^9 to 26.8 (±1.3) × 10^9.

Data from the metataxogenomic analysis (Table 5) clearly showed the very low diversity of the bacterial community from the bulk soil of the mining site (sample BSP). A total

Table 4 Numbers (colony forming units/g) of total viable heterotrophic microflora and total 16S rDNA genes in rhizosphere and soil samples (BSP, bulk soil at the mining site; PVP and DLP, rhizosphere soils of *Pteris vittata* and *Dicranopteris linearis*, respectively, at the mining site, and PVC, rhizosphere soil of *P.vittata* at the control site)

	TSA1/10	qPCR
	CFU × 10^6	number of copies × 10^9
PVC	39.5 (±9.69)	26.8 (±1.27)
PVP	46.6 (±13.9)	17.7 (±2.23)
BSP	0.0701 (±0.0506)	0.557 (±0.0680)
DLP	9.2 (±3.62)	10.5 (±5.65)

of 24,458 sequences were obtained and 11 phyla were detected in that soil with Proteobacteria and Nitrospira being very dominant (68 and 32%, respectively) (Fig. 4). The bacterial communities of the rhizospheres of *D. linearis* (DLP) and *P. vittata* (samples PVP and PVC) were more diverse whatever the taxonomic level considered, i.e., phylum, class, genus, or out, highlighting an important plant effect. On the other hand, there were only weak differences in terms of diversity between the non-polluted (PVC) and polluted (PVP) rhizosphere communities of *P. vittata*. As for the bulk soil, rhizosphere communities were consistently dominated by Proteobacteria. On the opposite, Nitrospira were detected at a much lower level. The other most abundant phyla within the rhizosphere community of *P. vittata* at the mining site were Acidobacteria (19%), Bacteroidetes (9%), Actinobacteria (6%), and Chloroflexi (5%). At the control site, the dominant phyla were Acidobacteria (14%), Bacteroidetes (12%), Chloroflexi (7%), and Planctomycetes (45%). In the rhizosphere communities of *D. linearis* (DLP), the five dominant phyla were Proteobacteria (34%), Bacteroidetes (17%), Actinobacteria (10%), Firmicutes (9%), and Chloroflexi (9%). Finally, based on diversity indexes (Table 5) as well as on prevalence of phylum/class/genus/OTU, there were more differences between the rhizosphere communities of the two plants collected at the mining site than between the rhizosphere of *P. vittata* at the mining site and that at the control site. Indeed, he H′ index of PVP and PVC sample were at similar level (8.24 and 8.58, respectively), whereas that of DLP sample was lower (5.98). The same trend was observed in the numbers of phylum/class/genus/OTUs.

The total sequence reads obtained for these four soils clustered into 10,796 OTUs and 11,911 OTUs, for PVP and PVC, respectively, and 140 OTUs and 4955 OTUs for BSP and DLP, respectively. Among the OTUs identified at a 3% divergence threshold, we looked then for the 50 dominant ones within each soil community. The *Acidithiobacillus* and *Leptospirillum* genera were highly dominant in the community of the mine BSP representing 67 and 32% of the dominant taxons, respectively. The comparison between the two rhizosphere communities of *P. vittata* showed the dominant genera to be Gp2, Gp3, and Gp4 from the *Acidobacteria* division, TM7 and *Rhizomicrobium* at the polluted site whereas the dominant ones were Gp4, Gp6, *Thiobacter*, and *Nitrosospira* at the control one. Some genera were only detected in the rhizosphere communities of *P. vittata* such as *Bacillus* (0.01% in PVP, 0.02% in PVC), *Cupriavidus* (0.12% in PVP, 0.01% in PVC), and *Pseudomonas* (0.05% in PVP, 0.34% in PVC). In addition, it has to be noted that several genera known to harbor opportunistic pathogen species were more abundant under metal pollution. For instance, *Burkholderia* was more prevalent in all polluted soils, PVP, BSP, and DLP than in the control one PVC. Similarly, *Ralstonia*, *Cupriavidus*, *Acinetobacter*, and *Mycobacterium* (Fig. 4) were more abundant in the rhizosphere community of *P. vittata* at the polluted site (PVP) than that of the control site.

Table 5 Summary of bacterial community estimators for the various soils. All the values were obtained from the subsampling of 24,458 sequences and at the sequence identity of 97% (cutoff value 0.03). (BSP, bulk soil at the mining site; PVP and DLP, rhizosphere soils of *Pteris vittata* and *Dicranopteris linearis*, respectively, at the mining site, and PVC, rhizosphere soil of *P.vittata* at the control site)

	PVP	BSP	DLP	PVC
Phylum	30	11	22	32
Classes	77	18	54	76
Genera	771	87	480	789
OTUs number	10,796	140	4955	11,911
Chao	59,508	518	23,338	90,472
Np Shannon H′	8.24	0.79	5.98	8.58

Discussion

Impact of metal pollution on *P. vittata* secondary metabolites

Our data demonstrated that metal pollution and more precisely Cu pollution resulted in modifications of secondary metabolite production in *P. vittata*. While chlorogenic acid derivatives were found to be significantly increased or decreased (depending on acetylation) in roots under heavy metal pollution, procyanidin was found in higher proportions when metals were present. This could be correlated with metal chelating ability of procyanidins which was reported previously (Karamać 2009; Mendoza-Wilson et al. 2016). Catechic tannins may be able to bind metal ions available in polluted soil such as Cu(II) or Pb(II), forming complexes thus allowing metal accumulation in roots. The concentration of Cu in some *P. vittata* roots from polluted areas are twofold higher than in the corresponding soils, whereas the Cu level in stems and leaves are much lower (data shown in Table S1) suggesting the accumulation of this metal in roots, but not in stems or leaves. These secondary metabolites were also shown to possess radical scavenging and antioxidant activities (Maldonado et al. 2005; Qiang et al. 2015) that could be useful to prevent the oxidative stress induced by metals (Sharma and Dietz 2009). Therefore, the overproduction of procyanidins in *P. vittata* roots could play a role in the adaptation process of this plant to metal pollution. The exudation of catechic tanin derivatives was also shown to affect bacterial denitrification processes in *Fallopia japonica* rhizosphere soil (Bardon et al. 2014). On the other hand, antibacterial activities were described for chlorogenic acid derivatives (Marques and Farah

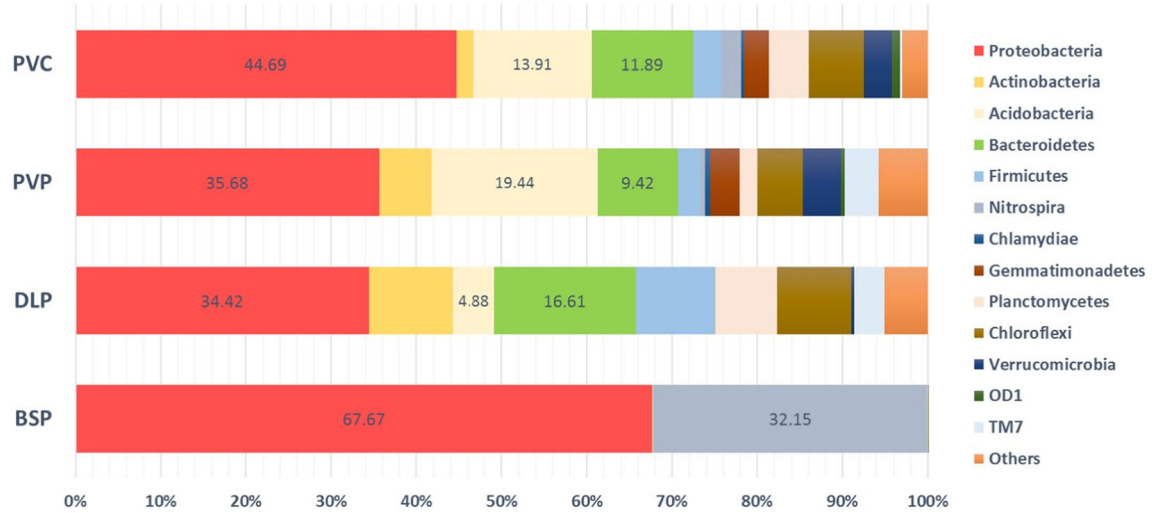

Fig. 4 Relative abundance of bacterial phyla from polluted and non-polluted sites (Illumina sequencing). From the mining site: PVP—soil under *P. vittata* polluted; BSP—bulk soil polluted; DLP—soil under *D. linearis* polluted. From the non-polluted site: PVC—soil under *P. vittata* control

2009; Lou et al. 2011). As only chlorogenic acid was increased in polluted sites and its acetylated derivatives decreased, all this suggests that the metal-induced changes observed in root metabolic composition could have an impact on the structure and functioning of bacterial communities from *P. vittata* rhizosphere. Whether these changes allow plants to recruit beneficial microbes that may help to adapt to this stress as hypothesized by Thijs et al. (2016) remains to be further investigated, but the fact that *Cupriavidus*, a bacterial genera known to be able to tolerate very high concentrations of Cu, was found in higher relative proportions in polluted soil could be a part of adaptation process of *P. vittata* under metal pollution.

In the aerial parts of *P. vittata*, while di-C-glycosylated flavones and 3-*O*-glycosylated flavonols (whether caffeoylated or not) were found in higher proportion under metal stress, only caffeoyl flavonol 3,7 diglycosides (quercetin- and kampferol-3-*O*-(caffeoyl)rutinoside-7-*O*-hexoside) were found to be decreased. This diminution may be due to the increase use of these compounds by the plant, including roots were some chlorogenic acid derivatives were found in higher proportions. Some of these metabolites (kaempferol and quercetin-3-*O*-rutinoside (rutin), kaempferol-3-*O*-glucuronide) were previously detected in *P. vittata* (Salatino and Prado 1998; Imperato 2000; Singh et al. 2008; Wahid et al. 2016). Moreover, the antioxidant properties of C-glycosyl flavones (Courts and Williamson 2015) and of some 3-*O*-glycosyl flavonols including rutin, kaempferol 3-*O*-rutinoside, and kaempferol 3-*O*-glucuronide were reported (Plumb et al. 1999; Yang et al. 2008; Leong et al. 2008; Ahmed et al. 2016). Besides, the preliminary 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay showed high antioxidant effect of *P. vittata* root, stem, and leave extracts (inhibitory percentage twofold higher than ascorbic acid at 100 μg/mL and similar at 500 μg/mL—data not shown). This led to hypothesis that the intensive production of these compounds in *P. vittata* could be a part of the antioxidant defense mechanism that limits reactive oxygen species (ROS) production and oxidative stress generated by heavy metals. The link between oxidative injuries generated by Cu^{2+} and variations of metabolic pathways is well documented in literature, and phenolics are mainly involved in cell protection (Dixon and Paiva 1995; Ali et al. 2006).

Overall, these results showed the influence of heavy metal contamination on the secondary metabolites of *Pteris vittata.* These polyphenols are well known for their properties against oxidative stress and seem to be involved in metal tolerance of *P. vittata*. But the impact of these changes in metabolism (especially in roots) on the structure of bacterial community profile has also to be considered.

Effects of metal and plant on diversity and composition of soil bacterial communities

The Ha Thuong mining site is particularly contaminated in Cu, with Cu level in bulk soil reaching more than 3000 mg/kg, ten times the accepted limit for industrial soils in Vietnam. Considering bacterial abundance, our data highlighted differences in the total heterotrophic microflora and the numbers of 16S rDNA copies. The most polluted soil (BSP) showed the lowest density in both cultivable bacteria and qPCR counts. This observation agreed with previous reports indicating the decrease of bacterial biomass resulting from metal contamination

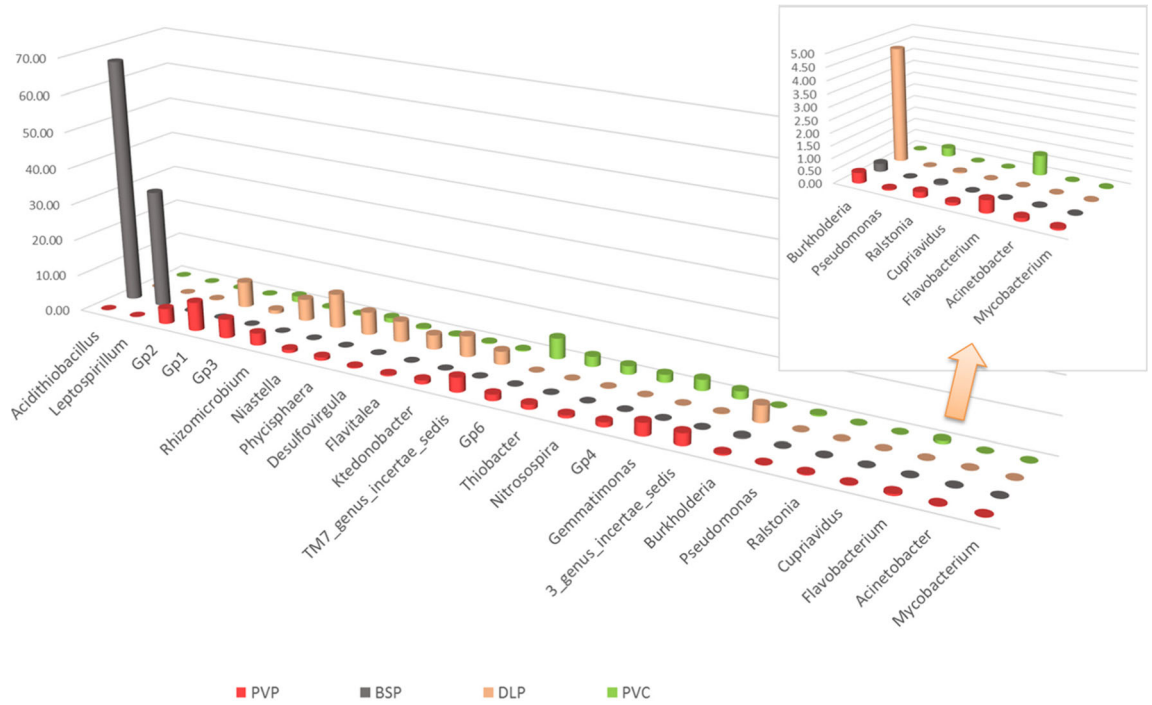

Fig. 5 Relative abundance (%) of dominant genus from polluted and non-polluted site. From the mining site: PVP—soil under *P. vittata* polluted; BSP—bulk soil polluted; DLP—soil under *D. linearis* polluted. From the non-polluted site: PVC—soil under *P. vittata* control

(Konopka et al. 1999; Kelly et al. 1999; Ekelund et al. 2003; Guo et al. 2009). However, in the rhizosphere samples, as metal contamination levels were moderated (PVP), or low (PVC, DLP), these variations are more related to differences in the amount of organic carbon in soil (mainly originating from the root exudates) than to amount of metals. The higher difference between culturable counts and qPCR ones in the bulk soil compared to rhizosphere soil could be related to a higher amount of uncultivable bacteria in this very acidic and oligotrophic environment.

Regarding bacterial community structure, our results showed a very low diversity and richness index of the community from the bulk soil at the mining site (Table 5). The diversity indexes Np Shannon and Chao and the prevalence of phylum/class/genus/OTU revealed that diversity and richness increased in the presence of plants as expected; however, there were more differences between rhizosphere communities of two plants from the mining site rather than between communities associated to *P. vittata* at the mining site and at the control one. This could be a consequence of the phytoextraction process, which leads to a strong decrease of Cu and Pb levels in planted soil and were not high enough (i.e., <450 mg/kg Cu, <300 mg/kg Pb) and bioavailable to affect bacterial communities. Wakelin and collaborators (2010) studied the impact of Cu on bacterial communities in a Cu-polluted field site in Hygum, Danemark, using denaturing gel electrophoresis (DGGE) and reported that bacterial diversity increased with Cu gradient but dramatically decreased at total Cu level above 560 mg/kg (Wakelin et al. 2010). Another work at the same site using high-throughput DNA sequencing of the 16S rRNA gene highlighted that the increase of Cu pollution did not impact the OTU richness (Berg et al. 2012). No definitive conclusion can be drawn from our results due to the low number of treated samples but one can suggest that Cu at very high level decreased the bacterial diversity and richness, whereas at lower concentration (below 450 mg/kg), the differences in the diversity of rhizosphere community are explained by plant influence rather than metal pollution.

Regarding bacterial composition, our data revealed that the bacterial community of the mining bulk soil consisted mainly of two genera, *Acidothiobacillus* (Proteobacteria phylum) and *Leptospirillum* (Nitrospira phylum). These genera were not detected within the three other communities. These observations are in agreement with the capacity of these genera to be able to thrive in oligotrophic and extremely acidic environments contaminated with heavy metals (Sánchez-Andrea et al. 2011; Zhang et al. 2016). At the phyla level, previous studies showed the relative abundance of Proteobacteria to be negatively affected following the increase of Cu level, for both total community (Berg et al. 2012) and transcriptionally active community (Nunes et al. 2016). However, it was also shown that the modification of bacterial community in the presence of Cu led to the increase of Proteobacteria relative abundance (Turpeinen et al. 2004). The dominance of Proteobacteria was shown to be a characteristic of metal contaminated soil whereas pristine soils are usually dominated by Actinobacteria and Acidobacteria (Sheik et al. 2012). In our study, Nitrospira is the second dominant phylum in polluted bulk soil but its

relative abundance was strongly reduced in rhizosphere samples from both plants (Fig. 5). This observation is in accordance with the result published by Nunes et al. (2016) indicating an increasing abundance of transcriptionally active Nitrospira along Cu gradient, suggesting that Nitrospira is a specific phylum dominating extreme Cu-polluted soil.

Our data showed that the OTU composition of the rhizosphere communities of *P. vittata* differed from that of the mine bulk soil. The dominant genera in the rhizosphere were Gp2, Gp3, Gp4, and Gp6 among Acidobacteria, *Rhizomicrobium*, *Thibacter*, *Cupriavidus*, *Bacillus*, and *Pseudomonas*. We also found that *P. vittata* select for different taxa in different soils. Some of these genera are known to prefer nutrient-limited environments (Hong et al. 2015) and are suggested to be bioindicators of Cu-related pollution in soils, i.e., Gp2, Gp3, Gp4, and Gp6 (Nunes et al. 2016). More interestingly, some genera as *Cupriavidus* were found at a higher level under metal pollution. Members of *Cupriavidus* genus are Gram-negative β-proteobacteria that are well known for their ecological role in heavy metal resistance, in particular *C. metallidurans* CH34 (Scherer and Nies 2009; von Rozycki and Nies 2009), *C. necator* N-1 (Poehlein et al. 2011), *C. taiwanensis* (Amadou et al. 2008), and *C. gilardii* CR3 (Wang et al. 2015). In addition, it has been demonstrated that some *Cupriavidus* species were also pathogenic such as *C. gilardii* (Karafin et al. 2010) and *C. metallidurans* (Langevin et al. 2011). Similarly, other genera known to harbor human opportunistic pathogens species including *Ralstonia*, *Acinetobacter*, *Burkholderia*, and *Mycobacterium* were also found to be enriched under metal pressure. Unfortunately, the taxonomic resolution achieved with our metagenomic strategy is not high enough to differentiate pathogenic species from non-pathogenic ones. Further studies are then needed to better understand the role of metal and/or plant secondary metabolites on the selection of specific taxa including opportunistic pathogens.

Conclusion

The present study showed a modification of *P. vittata* secondary metabolites, i.e., enhanced production of polyphenols known for their antioxidant properties, with heavy metal pollution, in particular Cu, and suggested the role of these discriminant metabolites in metal tolerance of this plant. Cu contamination level also altered the diversity and richness of bacterial communities in the mining bulk soil. In parallel, plant metabolism influenced the rhizosphere bacterial communities as some specific genera, of which *Cupriavidus*, *Acinetobacter*, *Ralstonia*, and *Mycobacterium* were increased in the rhizosphere of *P. vittata* at the polluted site. More detailed investigations are needed to understand whether the plant recruits beneficial bacteria to support the adaptation process to heavy metal stress, as supposed by Thijs et al., 2016. Similarly, our results suggested further work on the impact of secondary metabolites to soil bacterial communities including opportunistic pathogens.

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