

Intercropping-induced shifts in root microbiota promote lead phytoremediation properties of *Sonchus Asper*

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Abstract

Intercropping or assistant-endophytes has been proven to promote phytoremediation capacities of hyperaccumulators and to enhance their tolerance to heavy metals (HM) stress. We initially showed that intercropping with maize improved the remediating properties of the hyperaccumulator *Sonchus asper* (L.) Hill grown in HM contaminated soils, accompanied by an excluder - to- hyperaccumulator switched mode of action towards lead. To characterize molecular events underlying this shift in lead extraction strategy, we conducted an RNA-Seq analysis on *Sonchus* roots grown in intercropping or monoculture. We show that intercropping only slightly affects the *S. asper* transcriptome, but dramatically impacts the expression of root associated microbial genomes. Further, intercropping triggers a severe reshaping of endophytic communities which accompanies a “root-to-shoot” transition of lead sequestration and improved phytoremediation capacities of *S. asper*. This study provides the clue that a unitive network of plant- microbial -plant interactions may participate to the phytoremediation abilities of hyperaccumulator plants and paves the path for innovative cultural practices aiming to cure polluted soils.

Introduction

Heavy metal pollution of agricultural and land soils constitutes one of the most serious environmental issues worldwide, exemplified by the situation in China (Huang et al. 2018). China possesses large-scale mineral resources and is one of the largest global producers and consumers of metals (Li et al. 2014). Yet, long-term mining and smelting activities pose a high risk of toxic metal pollution, especially in southeast areas of the country (Yang et al. 2018). This may be illustrated by the Huize mining area, which is one of the typical representative areas of large-scale lead-zinc deposits in Yunnan province of China (Huang et al. 2003). Metal pollution causes deleterious damages on soil microbial communities and plant growth. Non-essential metals such as lead (Pb) have noxious effects, easily penetrate plant tissues and affect growth and general metabolism, like nutrient uptake and photosynthesis (Gopal and Rizvi 2008; Alves et al. 2014). So, removing heavy metals, from contaminated soils constitutes an intense field of research.

Phytoremediation has been proposed as a low cost, environment friendly and sustainable, effective method to restore soil integrity. This approach consists of using plants that extract and sequester metals from soils without obvious deleterious effects (Salt et al. 1998). Some herbaceous plants, such as *Arabis alpina* Var. *parviflora* Franch, *Cynodon dactylon* L. Pers, *Malva verticillata* L., and *Chenopodium ambrosioides* L., were found to grow naturally around lead-zinc mine tailing areas, that may accumulate diverse heavy metals (Li et al. 2019), (Zhan et al. 2019), (Mayerová et al. 2017), (Zhang et al. 2012) Besides, *Sonchus asper* (L.) Hill, another annual, herbaceous dicot which probably originates from the Mediterranean Basin (Hutchinson et al. 1984), is able to accumulate lead and cadmium, and is commonly distributed in Huize lead-zinc mining areas (Zu et al. 2005). Collectively designed as phytoremediators, these plants, among which *S. asper*, may be excluders, maintaining a relatively low concentration of metals in above-ground tissues compared to roots (Baker 1981) or hyperaccumulators, with a strongly enhanced rate of metal uptake and accumulation in leaves without any toxicity symptoms

(Rascio and Navari-Izzo 2011). Hyperaccumulators are of great interest, as they can be potentially used for phytoremediation (Verbruggen et al. 2009). However, hyperaccumulators may also have negative constraints of slow growth rate, low biomass production, and low restoration rate (Maestri et al. 2010). Therefore, it is necessary to combine the use of hyperaccumulators with other measures to improve the efficiency of heavy metal remediation (Chen et al. 2019).

Several studies reported the ability of hyperaccumulators to recruit beneficial microbes to enhance growth and tolerate environmental stress, including heavy metal stress (Miransari 2011). As a consequence, combining the use of hyperaccumulators and beneficial microorganisms to promote the phytoremediation processes appeared as a promising alternative biotechnology (Gupta and Joia 2016). This approach develops the association of assistant endophytes, rhizospheric bacteria and/or fungi to remove metals from contaminated soils through enhancing plant biomass production and facilitating phytoextraction or reducing phytostabilization (Glick 2010), (Ma et al. 2016), (Sharma et al. 2019). As an example, *Arabis alpina* was shown to host highly complex fungal and bacterial communities that actively contribute to increased heavy metal tolerance (Sharma et al. 2019), (Sun et al. 2019). More generally, dark septate endophytes (DSEs), a diverse group among ascomycetes (Jumpponen and Trappe 1998), and arbuscular mycorrhizal fungi (AMFs), which belong to the phylum Glomeromycota among Mucoromycetes (Brundrett and Tedersoo 2018), establish endophytic relationships with their hosts which result in improved extraction properties of the plant (Pawlowska et al. 2000; Torrecillas et al. 2013). They directly participate in the soil remediation process due to their ability to degrade organic contaminants and to decrease metal toxicity, in addition to their role as promoters of plant growth and metal accumulation and translocation (Ma et al. 2016; Zhan et al. 2019). However, an excess of metal concentration in soils may also be detrimental to the structure and dynamics of the rhizospheric microbiota.

Intercropping is a widespread cultural practice developed in Asian countries, especially in China (Knoerzer et al. 2010). This agricultural practice consists of growing two species next to each other, with an expected benefit on both partners in terms of improved nutrition and yield, as well as increased resistance to pathogens (Gaba et al. 2015), (Zhu and Morel, 2019). Intercropping is also developed between hyperaccumulators and other plants for remediation of heavy metal contaminated soils (Wu et al., 2007; Hussein et al., 2019). In a preliminary study, we found that the phytoremediation capacities of *S. asper* were enhanced when plants were grown under intercropping system with maize (*Zea mays*), a food crop widely planted in Yunnan Province (Pu et al., 2018). Other studies revealing that *Sonchus* roots are commonly infected by both DSEs and AMFs in natural environments (Massenssini et al. 2014) and that the soilborne microbial communities may be altered upon intercropping (Zhu and Morel 2019). These observations prompted us to hypothesize that *S. asper*, as an excluder, developed efficient ways to retain, sequester and possibly detoxify the metal within roots, maybe with the help of its associated microbiota. So, it became crucial to evaluate the impact of intercropping with maize on the global remediation properties, with a focus on eventual changes in the microbiota composition and on the global hyperaccumulator properties of *S. asper*.

To this aim, we investigated the effect of intercropping on the global transcriptome of the *Sonchus* root system using a RNA-Seq approach. We show in the present report that intercropping only slightly affects the *Sonchus* transcriptome, but dramatically impacts the composition and expression of the associated microbial genomes. Moreover, it is associated with a switch from an excluder to accumulator behavior towards lead, pointing out the possible contribution of the endophytic community of *S. asper* to the remediation strategy towards this metal. The study presented here illustrates efforts that have been made to connect studies on intercropping and investigations on the structure and dynamics of root associated microbiota, providing a new framework for studying plant-plant interactions in an enlarged concept of biotic partnerships.

Materials And Methods

Pot experiments in greenhouse and heavy metal content measurements

Some parts of the experiments conducted in the present study have been partially described in a previous report (Pu et al. 2018). Briefly, greenhouse experiments included two planting patterns (Supplementary figure S1): Monoculture of *S. asper* (MS) consisted of three plants in each pot, while a maize plant was placed between two *S. asper* plants in the intercropping (MS). A 10 cm intra-row spacing was designed between two plants in both planting systems. Three biological replicates were performed for each treatment. Pots were placed in a greenhouse with frequent irrigation to avoid drought stress, under a 12h/12h day/night photoperiod and a temperature $\leq 30^{\circ}\text{C}$. Plants were harvested from each replicate after 60 days. Collected samples were divided into two subsamples as follows: briefly, one half of *Sonchus* and maize root samples were washed with distilled water, frozen in liquid nitrogen, and stored at -80°C until RNA isolation. The rest of roots and shoot samples were used for determining biomass, heavy metal concentration, rhizosphere soil metal.

The concentrations of Pb in plants were measured from 0.5g of roots and shoots that were digested in $\text{HNO}_3\text{-HClO}_4$ (3/1, v/v) as described (Tanvir et al. 2014). The transport characteristics of Pb are expressed by translocation factor (Zu et al. 2005). The statistical significance of differences between Pb concentration among shoots, roots were determined by one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test ($p < 0.05$) using PASW Statistics 18 (SPSS Inc., Chicago, IL).

RNA isolation and RNA-Seq

Total RNA of *Sonchus* roots grown under monoculture (MS) or intercropping (IS) conditions was isolated using the Trizol reagent (Promega, USA) following the manufacturer's instructions, then treated with RNase-free DNase I (Takara Bio, Japan) for 30 min at 37°C to remove residual DNA. RNA concentration was measured using a 2100 Bioanalyzer at 260 nm and 280 nm, and only samples displaying a 260 nm/280 nm ratio between 1.8 and 2.0 were retained for subsequent analyses. Poly (A) mRNA was

isolated using oligo-dT beads (Qiagen). mRNA was fragmented (200 nucleotides) and reverse transcribed using random hexamers, followed by second-strand synthesis. Following purification, end reparation, poly (A) tailing and adapter ligation, cDNA fragments were purified by agarose gel electrophoresis and extraction of cDNA from gels, then enriched by PCR to construct the final cDNA library. Two cDNA libraries from each condition were sequenced on the Illumina sequencing platform (Illumina HiSeq™ 2500) using the single-end paired-end technology in a single run. The original images process to sequences, base-calling and quality value calculation were performed by the Illumina GA Pipeline (version 1.6), in which 100 bp paired-end reads were obtained (Li et al. 2013).

Bioinformatics analyses and sequence manipulation

To generate the unigene dataset, Illumina reads from the four libraries were mixed and assembled using Trinity (Haas et al. 2013), after removal of low-quality sequences, reads harboring more than 5% N (unknown) bases and reads containing untrimmed adaptors. The completeness of the transcriptome assemblies was assessed using BUSCO (Simão et al. 2015) with the eukaryotic gene set and the Viridiplantae gene set as references. Unigenes were ascribed to a given organism using a best hit BLAST approach against GenBank using the following criteria: query sequences matching nr hits with $\geq 70\%$ identity on $\geq 50\%$ of their length and an e value $\leq e-04$ were considered as belonging to a given organism, that was further classified in the tree of life using a non-hierarchical cladification system already published (Adl et al. 2019). Functional annotation included Blastn searches, as well as translation of unigenes and subsequent searches in NR and KOG/COG according to standard parameters. Transposable Elements (TEs) were searched by Blastn searches against the RepBase database using the Censor tool (Bao et al. 2015). Functional domains were predicted by alignment of ORGs of the unigenes against the PFAM (pfam.xfam.org) and PROSITE (<https://prosite.expasy.org>) databases. The presence of potential signal peptides was assessed using SignalP v5 (Almagro Armenteros et al. 2019). Prediction of transmembrane helices was performed at the HMMTOP server (www.enzym.hu). Nuclear export signals were searched at the NetNES server (cbs.dtu.dk). Fungal effectors were predicted with Effector2P (Sperschneider et al. 2016).

Determination of differentially expressed genes (DEGs)

Sample reliability analysis was conducted by estimation of the Pearson correlation. Expression levels of individual libraries were measured by the RPKM (Reads per kilobase transcriptome per million mapped reads) method (Mortazavi et al. 2008). Differentially expressed genes (DEGs) between MS and IS libraries were defined by false discovery rate (FDR), with P-values ≤ 0.001 and a \log_2 fold change $|\geq 2$.

Validation by qRT-PCR

To confirm the expression trends revealed by RNA-Seq, we performed qRT-PCR on a set of 14 genes. For the qRT-PCR validation, cDNAs were made from the RNA isolated by Trizol reagent (Invitrogen). Material was ground in liquid nitrogen, and total RNA was isolated using Trizol reagent. The integrity of total RNA was checked on agarose gels, and its quantity as well as purity were determined spectrophotometrically. A total of 50 ng to 2 μ g of RNA was used as a template for reverse transcription reaction in a 20- μ L

reaction volume using the FastQuant RT Kit (TIANGEN; www.tiangen.com). Real-time qRT-PCR was carried out using the qPCR Mastermix for TIANGEN. Reactions were run and quantification was performed on the ABI StepOnePlus Real-Time PCR Detection System (Applied Biosystems; www.thermofisher.com). PCR for each biological replicate was performed in three technical replicates. For each reaction, 2 μ L cDNA and 0.4 μ m primers (Supplementary Table S2) were used. The initial denaturing time was 2 mins, followed by 45 PCR cycles at 95°C for 5 s (denaturation), 60°C for 15s (annealing) and 72°C for 20s (elongation). The specificity of the amplification was confirmed by a single peak in a dissociation curve at the end of the PCR procedure. For each experiment, the stability of the reference gene across samples was tested using the Normfinder software (Andersen et al. 2004). Data were analyzed with RqPCRBase, an R package working on the R computing environment for analysis of quantitative real-time PCR data (Hilliou and Tran 2013). The differences were quantified in target gene expression between a standard control condition (MI). The mRNA levels were normalized against the constitutive expressed actin gene (Paolinelli-Alfonso et al. 2016). Amplifications for each biological replicate were performed in three technical replicates. To compare, gene expression variation direction from RNA-Seq and qRT-QPCR, Quantitative PCR values were transformed by log₂ Ratio (IS value/MS value).

Results

Intercropping improved phyto remediation transport characteristics of Sonchus asper towards lead.

Intercropping with maize led to a significant increase in Pb content in *Sonchus* plants. Refined analyzes revealed an unexpected change in the *S. asper* characteristics towards Pb, with a strong increase in lead content in aerial parts of the plants, concomitant with a reduced content in root tissues (Supplementary table S1). Consequently, the translocation factor increased from 0.8 to 1.8, resulting in a switch from excluder to hyperaccumulator behavior towards lead (Supplementary table S1). This confirmed the beneficial effect of the intercropping strategy for phyto remediation, previously evoked (Pu et al. 2018).

The RNA-Seq strategies unveils the nature of the *Sonchus* root-associated microbiota

To elucidate the possible causes of the observed excluder-to-hyperaccumulator switch in *Sonchus* upon intercropping, we intended characterizing the biotic community associated to the roots of *Sonchus* grown under monoculture and intercropping conditions. We hypothesized that changes in the composition and/or activity of the root microbiota upon intercropping would impact the bioremediation properties of the plant. Precisely, we suspected that intercropping with maize would induce a root-to-shoot translocation of lead through activation of transporters, and/or inhibition of the mechanisms responsible for the accumulation of the metal in roots in monoculture conditions.

We constructed several libraries from mRNA of *Sonchus* roots grown either in monoculture (MS) or under intercropping (IS) and generated RNA-Seq data. So, we expected to get a snapshot of the transcriptional

activity of plant tissues in the two situations, but also of the transcriptionally active biotic community possibly hosted in root tissues or around the root system. Sequencing yielded ~ 40 million, 125-bp long reads, of which 98.45% high quality clean reads for each library. In the absence of a *S. asper* genome sequence available, reads were pooled for *de novo* assembly using Trinity, that produced 65,357 contigs with an average length of 714 bp and N50 length of 1095 bp. We identified 297 of the 303 evolutionary conserved BUSCO eukaryotic genes, with only 1.98% of BUSCO genes missing from the assembly (Supplementary Table S3). Using the 430-gene plant dataset as a reference, the overall completeness score was 92.32% (Supplementary Table S3). So, the sequence coverage enabled further analyses. To evaluate the quality of the assembly, we checked for the actual occurrence of unigenes in the individual libraries and discarded 8595 sequences that were not represented by at least one read in at least one library. So, we retained a 56762-unigene dataset for the rest of the study. To identify the origin of the mRNAs corresponding to the unigenes, we performed a best hit Blastn analysis against nr hosted at GenBank. A total of 20339 unigenes could not be confidently ascribed to a given organism and were considered of 'unknown origin' (Supplementary Figure S2). As partial matches to GenBank entries or weak homologies could generate false positive results, we included additional parameters in the analysis, such as the percentage of identity, the length of the unigene matching an entry, the e value, as well as a minimal amount of reads in at least one library to estimate that the unigene is actually expressed and does not correspond to a contamination (see Materials and Methods). Retained sequences (16878 unigenes, Supplementary Figure S2) were largely of likely eukaryotic origin, but 22 unigenes better matched viral sequences, while 46 unigenes were of likely bacterial origin (Supplementary Table S4). Yet, it was not possible to know whether they derived from root-associated bacteria, or if these sequences are harbored by organellar genomes of eukaryotic hosts or if they have been acquired laterally. A further assignment of probable eukaryotic sequences revealed identified 91 fungal sequences and 200 unigenes of animal origin that had better analogs among nematodes, chordates and arthropods, to cite the most represented groups. So, although the sequences from likely plant origin constituted by far the most prominent proportion of the dataset (98.02%, Supplementary Table S4), the *Sonchus* root-derived transcripts might have diverse origins. This finding prompted us to compare the biotic, transcriptionally active assemblages in both monoculture and intercropping conditions.

Intercropping dramatically affects the composition of the *Sonchus* associated biotic community

Comparison was conducted through a two-step approach. We looked for genes that would be present in only one group of libraries and that would be considered as specific of a given cultivation condition, and we intended identifying differentially expressed genes (DEGs). Taken together, these searches revealed a total of 168 unigenes that were further analyzed, among which 76 "specific" genes and 92 DEGs (Supplementary Figure S3, Supplementary Table S5). Precisely, 15 unigenes were represented in the IS libraries and were absent from the MS libraries (Table 1, Fig. 1). They were assigned to diverse organisms, among which fungi (6), viruses (2), bacteria (2), nematodes (1), plant (1), while 3 unigenes remained of unknown origin. The 6 fungal genes better matched sequences from basidiomycetes, and mainly encoded proteins involved in stress responses and defense, such as ROS-scavenging enzymes,

heat shock proteins and stress responsive proteins (Table 1). The viral sequences showed significant homology with the coat protein from lettuce big-vein virus. The plant unigene corresponded to a partial sequence of a Copia transposable element. No open reading frame (ORF) of significant length could be deduced from the sequences of unknown origin.

We also identified 61 genes that were present in MS libraries and absent from the IS libraries. They were mainly from fungi (31 sequences, 51%), while only 15% were from plants and 21% could not be clearly assigned to an organism (Table 1, Fig. 1). Fungal genes were all confidently assigned to Glomeromycetes and could correspond to AMF inhabiting the *Sonchus* root system. Proteins could be predicted from all fungal unigenes, that could be classified in various KOG categories, despite the moderate number of genes (Table 1, Fig. 1). The most represented categories were “trafficking, secretion and vesicular transport (8/31), followed by “stress response and defense” (5/31) and lipid metabolism (4/31). This ranking came after manual annotation and the identification of 8 putative secreted proteins, among which 5 candidate effectors. This annotation step revealed also the presence of 3 putative secreted proteins and a Copia-like element among the open reading frames (ORFs) of unknown origin. Last, the eight sequences from plants also belonged to various KOG categories. Two of them encoded potential Copia-like elements. So, we could observe that intercropping induced an apparent transcriptional inactivation, if not extinction, of root-associated Glomeromycetes and the appearance of Basidiomycetes activating responses to stress.

We identified 37 Differentially Expressed Genes (DEGs) that were up-regulated upon intercropping and 55 down-regulated DEGs (Table 2, Fig. 2). They were mainly from plant (59%) and animals (22%), viruses and fungi being less represented (Fig. 2). Plant sequences corresponded to fragments of ribosomal DNA (rDNA) or mitochondrial DNA (mtDNA), despite a purification step of mRNA during the generation of libraries. Sequences from mtDNA mainly encoded various subunits of the NADH dehydrogenase complex, mainly reflecting an enhanced aerobic activity (Table 2). Unigenes from possible animal origin all matched nematode sequences, and derived from ribosomal RNA, at the noticeable exception to an unigene encoding a putative secreted effector of *M. incognita* which is over-expressed in parasitic stages of the nematode (Nguyen et al. 2018). Viral sequences mainly encoded RNA polymerase. The two fungal candidates were assigned to basidiomycetes. One of them encoded a potential membrane permease, while the other derived from ribosomal DNA. So, we could conclude that most up-regulated unigenes upon intercropping might be considered as ribosomal and mitochondrial contaminations of the RNA preparations.

Among the DEGs that were down-regulated upon intercropping, we found a majority (28/55, 51%) of plant genes, while fungal candidates and unassigned sequences each constituted 24% and 22% of the sample, respectively (Fig. 2). DEGs of likely plant origin were also classified into diverse KOG categories, where stress response and defense, protein turnover, transport, signal transduction and transposable elements constituted the best ranking (Table 2). Among the 13 genes of likely fungal origin, 4 unambiguously originated from ascomycete and mucoromycete donors, but the others did not display signatures of a particular fungal lineage. Together, fungal candidates encoded proteins classified in various categories,

including stress response, transport, cytoskeleton, signal transduction, but considering the very limited number of genes identified, no clear functional trend could be deduced from this annotation. Six out of the 12 DEGs of unknown origin contained ORFs that would encode putative secreted proteins, a protein containing a nuclear export signal, a Sec7 family protein, as well as 3 potential transposable elements. So, 6 DEGs remained without any taxonomic assignment and functional annotation (Table 2).

To validate the findings obtained from RNA-Seq data, we randomly selected 14 genes to confirm the observed effect of intercropping by quantitative real time (qRT)-PCR. We did not select genes that were up-regulated because they overwhelmingly derived from ribosomal and mitochondrial RNA, and primarily constituted contaminants from the RNA preparations. So, selected genes encoded various enzymes whose expression was lowered in RNA-Seq experiments. A significant, positive correlation between RNA-Seq and qRT-PCR data was observed for 11/14 genes (p-value < 0.05) (Supplementary Figure S4). It must be underlined here that the samples for qRT-PCR experiments were the same as those used for the RNA-Seq analysis.

Discussion

The present study shows that the excluder-to-hyperaccumulator switch accompanying the improved remediation properties of *S. asper* towards lead upon intercropping does not rely on gross modifications of the total plant transcriptome. Rather, our improved analysis of RNA-Seq data and comparison of RNA libraries from *Sonchus* roots grown in monoculture or in intercropping only identified 1% (168/16878) of an initial unigene dataset that displayed significant changes.

We first observed that the relative abundances of the major taxonomic categories are strikingly different within the total dataset and the set of genes submitted to transcriptional modifications. Hence, genes of plant origin, which constitute > 98% of the total unigene assembly, are particularly under-represented within the differential set, confirming that intercropping does not induce changes in the transcriptome of the plant itself. On the contrary, intercropping maize rather appears to dramatically impact the transcriptome of associated fungi and viruses.

We noted a lower expression of some plant genes associated to stress responses and transport upon intercropping. Whether it contributes to the change in accumulation characteristics of *S. asper* is not known. Strikingly, DEGs that are up-regulated upon intercropping mainly derive from ribosomal and mitochondrial RNA, whatever their organism of origin. These sequences might have been solely considered as contamination of the initial mRNA preparations and discarded from further analyses. However, they also testify from an increased biomass of the organisms from which they originate. This is in agreement with the initial proposition that intercropping stimulates *Sonchus* development (Pu et al. 2018). As well, the improved development of the root system would constitute a favorable niche to various members of the associated microbiota. In this context, *de novo* assembly of RNA-Seq reads and further taxonomic assignments revealed unigenes that displayed a best hit against Genbank entries from animals, including chordates and arthropods. This finding was not surprising, as we could anticipate

finding traces of insects, rodents, and other small animals at the vicinity of *Sonchus* roots. They are absent of the differential dataset, as they likely constitute a natural contamination of RNA from soil. The presence of nematodes, which are major plant pathogens that attack a myriad of plant families, including *Sonchus* (Jones et al. 2013), was also expected. Their overrepresentation in DEGs up-regulated upon intercropping may reflect an increased plant susceptibility to root pathogens, as a possible consequence of the observed decreased stress responses of the root tissues.

The unexpected over-representation of fungal genes has to be re-evaluated, as different phyla are represented in various situations. Hence, Basidiomycetes emerge upon intercropping, while Ascomycetes and Glomeromycetes which are by far the most represented in monoculture, are absent in IS libraries. Specific explorations of the root system of *Sonchus* grown in monoculture and in intercropping are necessary to conclude whether mixing *Sonchus* and maize actually leads to a transcriptional inactivation of these fungi or to their extinction. Nonetheless, the present finding is of importance. *Sonchus* roots are known to be inhabited by a diverse fungal community consisting of both dark septate endophytes (DSE) and arbuscular mycorrhizal fungi (AMFs), which belong to Ascomycetes and Glomeromycetes, respectively (Pendleton and Smith 1983), (Knapp et al. 2012). The predicted presence of these phyla is supported by the taxonomic assignment of unigenes present in monoculture, but not intercropping libraries. Here again, their possible extinction may suggest, along with a rise of basidiomycetes akin to the plant pathogen *Rhizoctonia solani*, the increased presence of pathogenic nematodes, and the diminished expression of plant responses to stress, that intercropping indirectly leads to an increased plant susceptibility to pests. We can also suppose that the elevated phytoextraction efficiency provided by intercropping results in a reduced toxicity of the soil, which offers more favorable conditions for the re-shuffling of biotic interactions and a new composition of the root-associated microbiome.

Our initial description of the excluder-to-hyperaccumulator changed characteristics of *Sonchus* towards lead upon intercropping, which is confirmed in the present study, relied on the increased Pb translocation factor that resulted from the concomitant rise of Pb concentration in shoots and a decrease in roots. These results, in frame with the observed significant decrease of the effective Pb in the rhizosphere soil (Pu et al. 2018), lead us to rise the hypothesis that the excluder characteristics of *Sonchus* towards lead are actually assumed by its endophytic fungal community and that intercropping, through the possible breakdown of this association, restores a basic 'accumulator phenotype' otherwise observed for other heavy metals such as cadmium (Pu et al. 2018). This hypothesis relies on the following observations. First, it is known that structures developed by DSEs and AMFs may constitute a physical barrier that absorb and retain heavy metals and consequently lead to a reduction of metal root-to-shoot translocation, as a potential protection of shoot tissues against metal damage (Zhan et al. 2019). This would be illustrated by the important abundance of Glomeromycete genes in the MS libraries. Second, a significant proportion of these genes encode secreted proteins, among which potential effectors, proteins that are secreted by pathogenic microorganisms to defeat defenses and manipulate cellular functions of their hosts to achieve infection (Khan et al. 2018). They are also used by symbiotic fungi for successful plant colonization (Kamel et al. 2017). Their absence in the IS libraries or in the up-DEGs would illustrate the symbiosis disruption and the consequent activation (or suppression of the inhibition) of the root-to-

shoot translocation of Pb in a transcription-independent manner. The suppression of endophytic fungi would also explain the global decreased lead concentration in *Sonchus* roots upon intercropping.

If the hyperaccumulator characteristics of *Sonchus* are due to the suppression of endophytic fungi upon intercropping, it suggests that maize is responsible for this phenomenon. A possibility is that intercropping induces the attraction and subsequent migration of the fungal community from *Sonchus* towards maize roots. Endophytes may be eliminated by *Sonchus* itself, through a decreased tolerance of the plant to its microbiote. We can also suppose interactions between the *Sonchus*-associated microbiome and the biotic community associated to maize roots and the establishment of antagonistic interactions among this wealth of microorganisms, leading to a decrease or suppression of the *Sonchus* endophytes. Last, a likely hypothesis is that maize root exudates contain molecules that may be toxic to *Sonchus* associated fungi or would destabilize the symbiotic associations. Several studies aiming at defining the composition of maize root exudates revealed that benzothiazole (BZO), an aromatic heterocyclic compound, as well as benzoxazinoids, like 2,4-Dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and its degradation product 6-methoxy-benzoxazolin-2(3H)-one (MBOA) possess antimicrobial activity against plant pathogenic fungi and oomycetes (Yang et al. 2014), (Mei et al. 2019). Other molecules, like phenolic acids, were found in root exudates and have a strong antimicrobial activity against these microbial pathogens (Zhu and Morel 2019), (Zhang et al. 2020). As a result, maize may be considered as a good helper to improve the remediation abilities of hyperaccumulator *S. asper* through intercropping. In addition, identifying the components of the maize root exudates and assessing their potential antifungal activity against endophytes and AMFs of *Sonchus* would be a first step towards unraveling the mechanisms described in the present study.

Conclusion

Intercropping triggered an “excluder - to - hyperaccumulator” mode transition of *Sonchus asper* towards lead when planting with maize, but the fundamental mechanisms underlying the improved phytoremediation efficiency of *Sonchus* are still largely unknown. The present study reveals that intercropping-induced shifts among the plant-associated microbiota communities may contribute to a “root-to-shoot” transition of lead transportation and sequestration. Moreover, it speculates that maize root-derived exudates may play an important (positive and negative) role in reshaping this microbial community. These results contribute to the emergence of a new conceptual framework that favors the simultaneous study of plants and biotic associates to explain mechanisms underlying heavy metal tolerance and remediation of polluted soils. Last, combined microbiota-assisted bioremediation with intercropping could be considered as a valuable method for remediation of contaminated soils.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

Not applicable

Competing interests

The authors declare that they have no conflict of interest.

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Authors' contributions

ZYQ, ZFD, LZR and HYM designed the research; PLL performed part of the research; AP and MdR designed and conducted bioinformatic analyses; ML analyzed qPCR data and performed statistical analyzes; MXY and FP analyzed and interpreted the data and wrote the paper. WY contributed to the writing of the paper. All authors read and approved the final manuscript.

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Tables

Due to technical limitations, the tables can be found as a download in the supplementary files section.

Figures

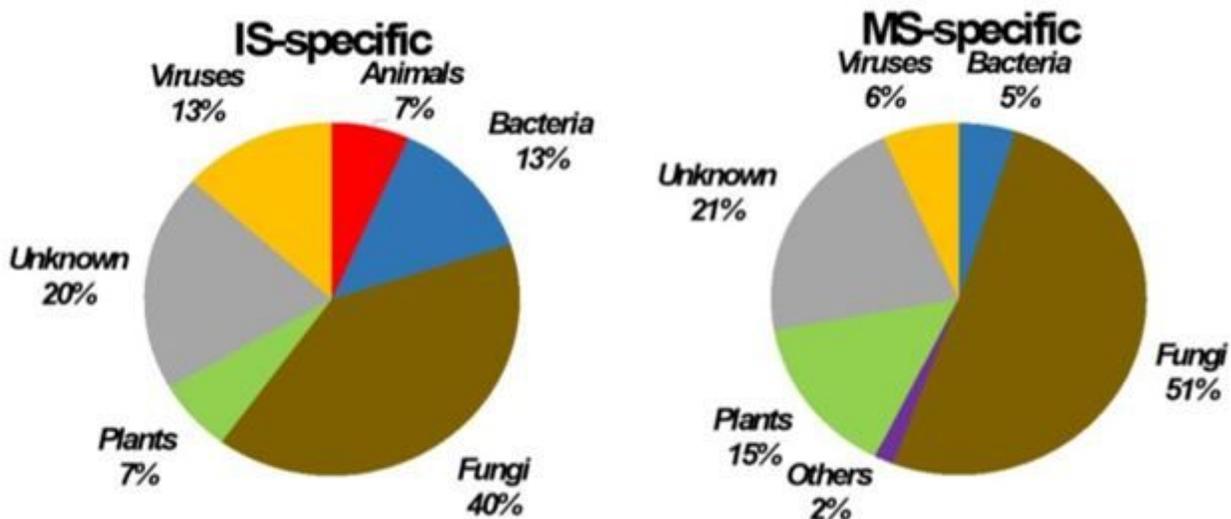


Figure 1

Taxonomic assignment of the most significant DEGs from *Sonchus* roots that are up-regulated upon intercropping with maize.

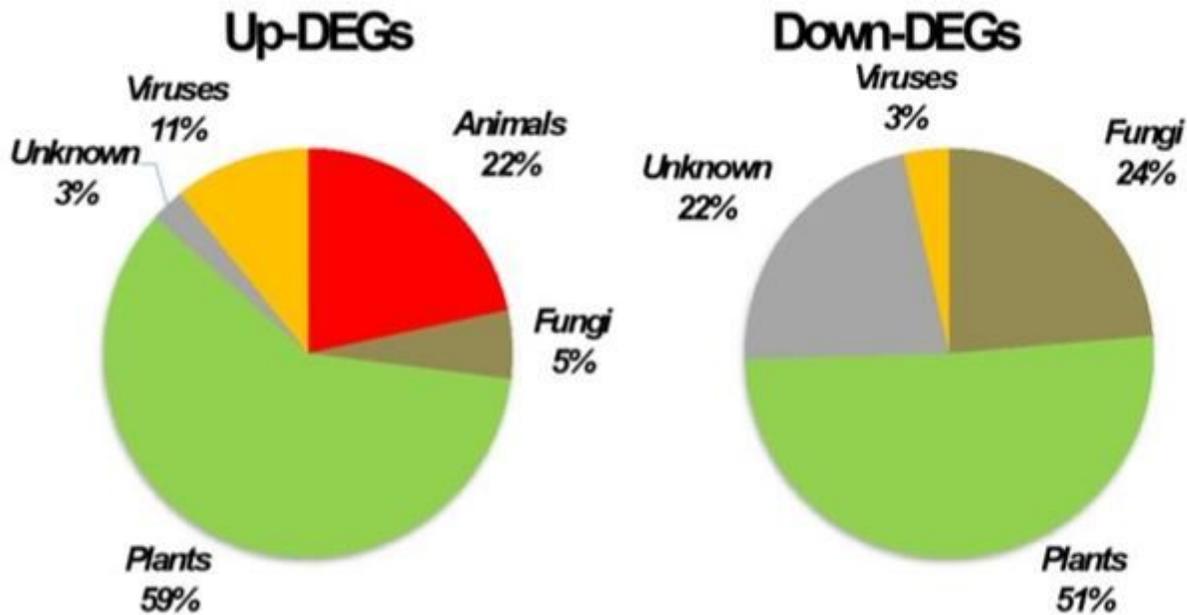


Figure 2

Analysis of the *Sonchus* root transcripts that are down-regulated upon intercropping with maize. A: Taxonomic assignment the DEGs that are present in MS libraries and absent from IS libraries. B: Functional annotation of the genes of potential fungal origin according to the Eukaryotic orthologous group (KOG) classification. Transposable elements constitute an additional category (AA). C: Taxonomic assignment the DEGs that are actually down-regulated upon intercropping. D: KOG classification of the unigenes from plants.

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