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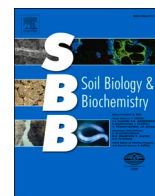
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Rhizochemistry and soil bacterial community are tailored to natural stress gradients

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ABSTRACT

Plants modulate their rhizochemistry, which affects soil bacterial communities and, ultimately, plant performance. Although our understanding of rhizochemistry is growing, knowledge of its responses to abiotic constraints is limited, especially in realistic ecological contexts. Here, we combined predictive metabolomics with soil metagenomics to investigate how rhizochemistry responded to environmental constraints and how it in turn shaped soil bacterial communities across stress gradients in the Atacama Desert. We found that rhizochemical adjustments predicted the environment (*i.e.* elevation, R^2 between 96% and 74%) of two plant species, identifying rhizochemical markers for plant resilience to harsh edaphic conditions. These metabolites (*e.g.* glutamic and succinic acid, catechins) were consistent across years and could predict the elevation of two independent plant species, suggesting biochemical convergence. Next, convergent patterns in the dynamics of bacterial communities were also observed across the elevation gradient. Finally, rhizosphere predictors were associated with variation in composition and abundance of bacterial species. Biochemical markers and convergences as well as potential roles of associated predictive bacterial families reflected the requirements for plant life under extreme conditions. This included biological processes such as nitrogen and water starvation (*e.g.* glutamic and organic acids, Bradyrhizobiaceae), metal pollution (*e.g.* Caulobacteraceae) and plant development and defence (*e.g.* flavonoids, lipids, Chitinophagaceae). Overall, findings highlighted convergent patterns belowground, which represent exciting insights in the context of evolutionary biology, and may indicate unique metabolic sets also relevant for crop engineering and soil quality diagnostics. Besides, the results emphasise the need to integrate ecology with omics approaches to explore plant-soil interactions and better predict their responses to climate change.

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1. Introduction

The ability of a plant to thrive in an ecosystem is closely dependent on autonomous (e.g. genetic make-up) and non-autonomous capacities (e.g. interactions with soil bacterial communities and other species) (Kraiser et al., 2011; Trivedi et al., 2020; van Dam, 2009). Plants alter soil properties and drive soil microbiome and rhizosphere interactions via root exudates (Bennett and Klironomos, 2019; Berendsen et al., 2012; Oburger and Schmidt, 2016; Vismans et al., 2022; Yu et al., 2021). In turn, the soil bacteria influence the soil structure and can also positively or negatively influence plant performance (Gibert et al., 2019; Hartmann and Six, 2022; Jones and Smith, 2004). Besides, interactions between plants and soil microorganisms were recently shown to act as a key driver of plant adaptation (Cosme, 2023). The rhizosphere, which refers to the area around a plant root (Cotton et al., 2019; Hartmann et al., 2008; McLaughlin et al., 2023), is the nerve centre of intense chemical dialogue between the plant and the soil bacterial communities (Badri and Vivanco, 2009; Bennett and Klironomos, 2019; Cotton et al., 2019; Fitzpatrick et al., 2020). Analysing rhizochemistry is complex since it includes plant exudates, their breakdown products as well as bacterial compounds (Pétriaccq et al., 2017). For instance, plants release organic acids to improve resource uptake or tolerance to soil toxicity (Carvalhois et al., 2011; Frémont et al., 2022). Plants exude a wide diversity of compounds from different classes, such as flavonoids, coumarins and lipids to condition bacterial communities, and some metabolites, such as benzoxazinoids, can be metabolised by microorganisms into antimicrobial compounds (Cotton et al., 2019; Luginbuehl et al., 2017; Macías et al., 2005; Sasse et al., 2018; Vismans et al., 2022; Yu et al., 2021). Thus, analyses of non-sterile rhizosphere should be used as a holistic approach to capture a more complete fraction of rhizosphere signals, instead of plants isolated from their natural environment (Pétriaccq et al., 2017; Schandry and Becker, 2020; Steinauer et al., 2023). Overall, recent efforts improved our understanding of some biochemical mechanisms used by plants to interact with the microbial community (Hawkins et al., 2023). Nevertheless, it is reasonable to say that a significant portion of rhizochemicals remains to be discovered and that little is known about how these signals shape the surrounding microbe community (Delory et al., 2016; McLaughlin et al., 2023; Pétriaccq et al., 2017). Moreover, most studies were performed on single species. Hence, rhizosphere chemicals should be explored in wild ecosystems and across multiple plant species to gain a more realistic image of soil chemistry *in natura*.

Soil bacterial communities and plant rhizochemistry are influenced by abiotic constraints (Baldrian et al., 2023; Bennett and Klironomos, 2019; Mandakovic et al., 2023). Previous studies detailed the effects of abiotic stress such as drought on mycorrhizal fungi, plant exudates and plant-microbe interactions (Branco et al., 2022; Del Valle et al., 2020; Gargallo-Garriga et al., 2018; Ruiz-Lozano et al., 2016; Trivedi et al., 2022). Interestingly, the response of soil chemistry to environmental pressures such as drought seems highly variable between plant species and environments (Trivedi et al., 2020). However, the plant species- or environment-specific character of most studies may explain this high specificity. Besides, most experiments were conducted under controlled conditions, which only enabled the analysis of short-term responses. In contrast, studies in wild ecosystems offer a unique opportunity to explore the responses of rhizochemistry to abiotic stress that result from long-term adaptation processes.

The Atacama Desert is the driest non-polar desert on Earth (Eshel et al., 2021). In this desert, the Talabre-Lejía transect (TLT) is an elevation gradient (≈ 2500 – 4500 m.a.s.l.) characterised by extreme scarcity of water (20 – 160 mm.yr⁻¹), nutrient deprivation, and high solar irradiance (600 W m⁻².d⁻¹) (Eshel et al., 2021). Tens of plant species thrive in this transect and define three plant communities: the Prepuna (2500 – 3300 m.a.s.l.), the Puna (3300 – 4000 m.a.s.l.) and the Steppe (4000 – 4500 m.a.s.l.) (Díaz et al., 2019). Thus, the Atacama Desert represents an ideal system to study responses of the

rhizochemistry to this gradient of extreme abiotic constraints. Besides, a high variation in nature and abundance of soil microorganisms has previously been reported for this area (Eshel et al., 2021; Mandakovic et al., 2023; Bartholomäus et al., 2024). Here, we addressed whether and how rhizochemicals and associated bacterial communities responded to a linear variation in abiotic constraints across the elevation gradient. In addition, we investigated the specificity level of this response across plant species. As a previous study highlighted convergent responses in multiple chemical families aboveground (Dussarrat et al., 2022), we hypothesized that inter-species similarities might also occur belowground at the chemical and bacterial community level. To answer these questions, we collected four plant species and the associated rhizosphere and bulk soil fractions at various elevations across the entire gradient to explore the rhizochemistry responses to the different abiotic factors. In addition, we used previous sequencing data in operational taxonomic unit (OTU) abundances across the transect (Mandakovic et al., 2023) to investigate whether convergence was also observed in the response of bacterial communities. Finally, subsequent bioinformatic analyses were applied to link rhizosphere metabolic predictors to the composition of the associated bacterial communities across the gradient.

2. Materials and methods

2.1. Sampling

Non-sterile rhizosphere samples were collected in the Talabre-Lejía transect (lat 22°–24°S) in April (7 and 8) 2021 at 5 cm depth under four distinct plant species, whose aerial parts were also sampled (Table S1). The sampling period (after the rainy season) and sites were selected based on previous studies that have proved effective in capturing plant biodiversity and biochemical diversity aboveground, while a 5 cm depth was selected as important processes occur at this depth such as organic matter degradation and soil oxygenation, among others (Díaz et al., 2024; Dussarrat et al., 2022). For each rhizosphere sample, the soil was dug to a 5 cm depth under the plant and the surrounding rhizosphere was homogenised using a small shovel. Then, 5 mL of soil were collected in an Eppendorf tube and directly snap-frozen in liquid nitrogen. Any visible root fragments in rhizosphere samples were removed. Species included *Atriplex imbricata* (Moq.) *D.Dietr.* (Amaranthaceae), *Hoffmannseggia doellii* Phil. (Fabaceae), *Adesmia spinosissima* Meyen ex Vogel (Fabaceae) and the Poaceae *Jarava frigida* (Phil.) F. Rojas (synonym of *Pappostipa frigida* (Phil.) Romasch). Each pair of samples (*i.e.* rhizosphere and associated plant), was collected in 1–4 elevation sites (Fig. 1), depending on species availability, with a minimum of three biological replicates for both rhizosphere and plant samples. In addition, two soil samples were taken from open areas (*i.e.* without plants aboveground) within a ≈ 30 cm radius around the target species at each elevation. When two species were collected at a given altitude, open-area samples were collected between the target species. Rhizosphere samples under *J. frigida* and plant samples from *A. imbricata* and *J. frigida* were also collected in April (6 and 7) 2019 (Table S1). Rhizosphere samples of *J. frigida* collected in 2019 included sites collected in 2021 (4,270, 4,072, 3870 m.a.s.l.), as well as sites that were not sampled in 2021 (4480 and 3970 m.a.s.l.). Frozen samples were transported to the laboratory in dry ice, freeze-dried and ground. Dried sample powders were stored at -80 °C until extraction.

2.2. Environmental data

The environmental conditions along the elevation transect, characterised in a previous study, were represented by chilling to freezing temperatures during the night, as well as extremely low annual rainfall and high daytime solar radiation levels compared to the global average (Eshel et al., 2021). Over the years, climatic data were assessed every hour using two meteorological stations (3060 and 4090 m.a.s.l.). Soil composition was previously obtained and described for over 3 years

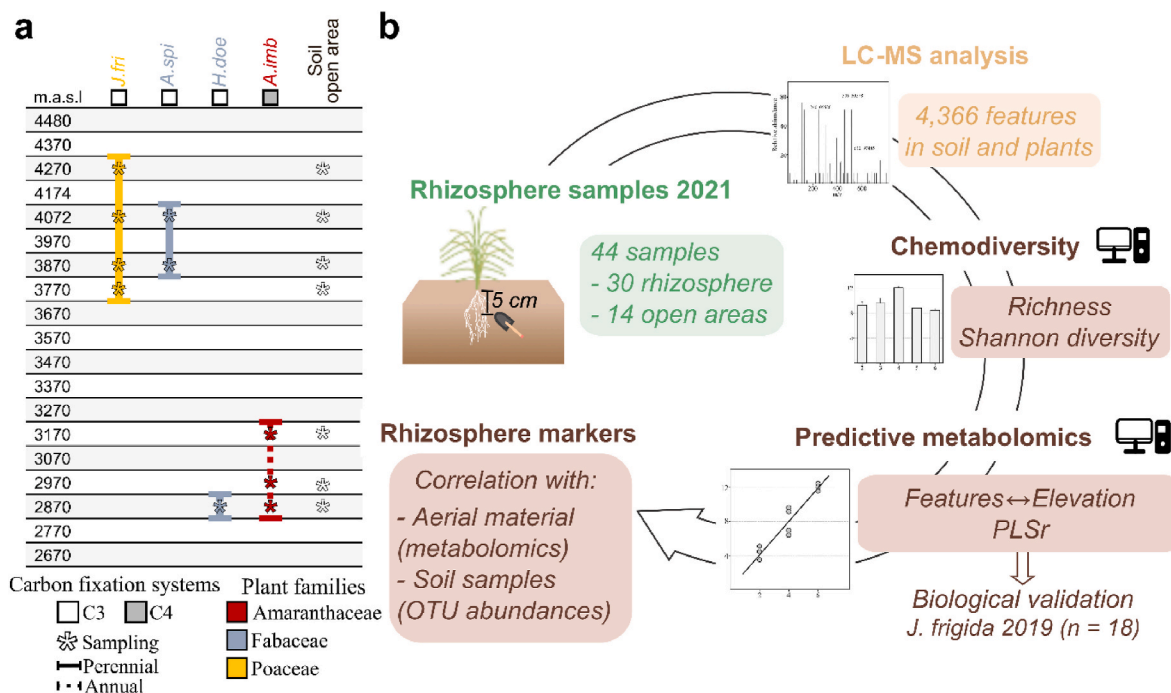


Fig. 1. | Sampling and analytical workflow. **a.** Sampling of plant and rhizosphere material. **b.** Simplified workflow of the analysis. *A.imb*: *Atriplex imbricata*, *A.spi*: *Adesmia spinosissima*, *H.doe*: *Hoffmannseggia doellii*, *J.fri*: *Jarava frigida*.

without major changes (Eshel et al., 2021). Measured climatic and edaphic parameters included: temperature, humidity, solar irradiance, precipitation, soil water content (here preferred over precipitation as it accounts for snow level at high elevations), pH, as well as nitrate, ammonium, Olsen phosphorous, zinc, potassium, manganese, copper, iron, boron, molybdenum, sulphur, calcium, manganese, sodium, chlorine, bicarbonate salt and silt contents. Values of these parameters are indicated in Dussarrat et al. (2022) and in Table S1 of this manuscript.

2.3. Soil bacterial communities

Previously, the relative abundance of OTUs had been assessed in both bulk soil (BS) and rhizosphere surrounding samples (RSS) under multiple plant species, each at a given altitude, through the Atacama transect (Mandakovic et al., 2023). In this previous study, the bacteria-specific primer set 28F (5' GA GTT TGA TCM TGG CTC AG 3') and 519R (5' GWA TTA CCG CGG CKG CTG 3') with a barcode in the forward primer were used to amplify the bacterial 16S rRNA gene. Samples were sequenced by Molecular Research LP (MR DNA) on an Illumina MiSeq platform. Sequence data processing included the removal of barcodes and sequences <150 bp, a grouping of valid sequences with 4% divergence using Usearch (v6.1.544) and a filtration step of sequences with a minimum quality of q30 (Mandakovic et al., 2023). For this present study, OTU data from each dataset (BS and RSS) were rarefied to 8000 reads and only OTUs identified at the family level were used (10,515 OTUs) to test for changes in the composition of the soil bacterial community in response to elevation.

2.4. Metabolomics

To maximise the extraction of semi-polar compounds, we performed robotised ethanolic fractionation using 450 mg or 20 mg of dried soil and plant material, respectively (Luna et al., 2020). Ethanol extracts were subjected to ultra-high-pressure liquid chromatography (UHPLC) - Orbitrap mass spectrometry analysis using an Ultimate 3000 UHPLC combined with an LTQ-Orbitrap Elite MS (ThermoScientific, Bremen, Germany). The separation was realised on a C18 column (C18-Gemini,

150 × 2 mm, 3 μm, 110 Å, Phenomenex, France) coupled to a C18 SecurityGuard Gemini pre-column (4 × 2 mm, 3 μm, Phenomenex, France) at 30 °C with an injection volume of 5 μL, a flow rate of 350 μL/min and a previously established gradient (Luna et al., 2020). The LTQ-Orbitrap was operated in negative electrospray ionisation (ESI⁻) mode (Dussarrat et al., 2022; Luna et al., 2020). MS spectra were acquired using data-dependent analysis at a resolution of 30,000 in a *m/z* range of 50-1500. Quality controls (QC) were injected every 10 samples.

Raw LC-MS data were processed via MS-DIAL (v. 4.90) using optimised parameters (Tsugawa et al., 2015) detailed in Table S2. Putative annotation was performed using the MS/MS-Public-Neg database (v. 17). Feature filtering was performed using blanks and QC samples, where features exceeding a coefficient of variation of 30% were excluded. The resulting dataset (4629 features) was first normalised by sample weight, followed by median normalisation, cube-root transformation and Pareto scaling using MetaboAnalyst (v. 6.0) (Xia et al., 2015) before statistical analyses. The non-normalised dataset obtained after preprocessing is available in Table S3 and was deposited online (see Data availability).

2.5. Annotation and classification

The automatic annotation (see above) was complemented by a manual putative annotation for the best predictive features as previously described (Dussarrat et al., 2022). Putative chemical formulas were defined by screening accurate *m/z* on MetFrag (Ruttkies et al., 2016), and the putative annotation was performed using ChEBI (Hastings et al., 2016) and KNApSACk (<http://www.knapsackfamily.com/KNApSACk/>) databases. MS/MS spectra were compared to experimental spectra on Massbank (Horai et al., 2010). The metabolomics standards initiative levels (MSI levels) were used to define the confidence level in the putative annotations (Sumner et al., 2014), as previously performed (Dussarrat et al., 2022, 2023). All annotations should be considered putative, even when confirmed with an MS/MS match (MSI 2 level). Biochemical classes and pathways were defined using ChEBI and ClassyFire taxonomy (Djoumbou Feunang et al., 2016).

2.6. Statistical analyses

The normalised metabolic dataset was processed through multivariate analysis using MetaboAnalyst (v. 6.0) and R software (v. 4.2.1) (R Core Team, 2022; Xia et al., 2015). Principal component analysis (PCA) was performed using *FactoMineR* package (Lê et al., 2008). Variation in chemical diversity was explored using the *chemodiv* package to calculate richness and Shannon's indices (Petrén et al., 2023). To extract rhizosphere or plant features responding significantly to elevation, we performed ANOVA tests in MetaboAnalyst and tested Pearson's correlation using the *Hmisc* package (Harrel, 2020). Features responding significantly to elevation and significantly correlated with elevation (Pearson's correlation, $P < 0.05$, FDR correction) were extracted for subsequent analyses. However, for *A. imbricata*, the 54 features showing a significant trend (i.e. $P < 0.05$ without FDR correction) were also kept as only 8 features were significant with FDR correction. This threshold of $P < 0.05$, FDR correction is universal and well-suited for metabolomics analyses (Dussarrat et al., 2022), although we recognise that some features of interest might be excluded despite great correlation with elevation. To test the capacity of rhizochemistry to predict plant elevation, we performed partial least squares regression analyses (PLSr) via the *pls* package, which was used to select the optimal number of components ("onesigma" function, one component was selected) and to perform the predictions (Liland et al., 2022). Sample sets were divided into a "training" (80%) and a "testing" set (20%) using stratified sampling. This optimised 80/20 ratio is widely accepted (Dussarrat et al., 2022), but is not indicative of the robustness and generalisability of the model, which are ensured by permutation tests and an independent validation set, as described below. Models were performed 50 times to cope with random partitioning and prediction quality was assessed by fitting predicted elevations to measured values. To test the likelihood of spurious predictions, 50 permutation sets were created for each model by randomly swapping elevation levels between samples using the *transform* function in R (v. 4.2.1) as previously described (Dussarrat et al., 2022). Next, the predictive capacity of the predictive features (hereafter referred to as markers) was confirmed by performing real predictions on independent datasets (i.e. independent data not included in either the training or the testing set). Model equations were developed on plants (*A. imbricata* or *J. frigida*) collected in 2021 and directly applied to either (i) the other two species (*H. doellii*, *A. spinosissima*) and (ii) plants of the same species collected in 2019. Tukey's tests were performed to compare the accuracy of the models using the *agricolae* package (Mendiburu, 2020). Figures were designed using *ggplot2*, *ggpubr* and *Hmisc* packages (Harrel, 2020; Kassambara, 2020; Wickham, 2016).

Subsequent statistical analyses were performed to link rhizosphere metabolic predictors with aboveground metabolism. We first extracted metabolic features that responded significantly to elevation in aboveground samples of *A. imbricata*, *A. spinosissima* and *J. frigida* collected in 2021 and 2019 (Tukey's or Student's test, $P < 0.05$, FDR correction). We then tested whether rhizosphere metabolic predictors were also responding significantly to elevation in aerial tissues (Fig. S1).

Next, the intensity of the rhizosphere markers was linked to changes in the composition of the soil bacterial community (Fig. S1). We extracted OTUs responding significantly to elevation ($P < 0.05$, FDR) and detected in at least three RSS samples to exclude OTUs found under specific plant species or genera, yielding a set of 1973 OTUs. To test for convergent responses of bacterial communities to elevation, PLSr models were performed as detailed above (see 2.4 Statistical analyses). Real predictions were performed by excluding four species from the RSS dataset (*Exodeconus integrifolius*, 2870 m.a.s.l.; *Trichoclina caulescens*, 3370 m.a.s.l.; *Lupinus subinflatus*, 3870 m.a.s.l.; *Calamagrostis cabrerae*, 4270 m.a.s.l.). Model equations were developed on the remaining dataset and directly applied to these four species to predict their elevation. The most predictive OTUs (top 1%, 105 OTUs) were defined based on their coefficients in the models. Putative functional roles of the top 1%

predictive OTUs were assigned via the *PICRUSt2* package (Douglas et al., 2020) using 16S rRNA sequences and the KEGG database as previously described (Mandakovic et al., 2023).

We then linked the response of plant rhizochemistry and bacterial communities. According to our results, convergent responses of plant rhizosphere to elevation might be observed, which could lead to similarities in the response of certain bacterial species. In this context, correlations between OTU abundances and metabolic intensities were tested regardless of the plant species under which samples were collected. For Steppe species, the average intensity of the best 216 markers was calculated for each elevation at which *J. frigida* or *A. spinosissima* were collected in both experiments (i.e. 3,870, 4,072, 4270 m.a.s.l., Fig. S1). OTU abundances of RSS samples at the same elevations were extracted (30 samples, 10 plant species, 3 elevations) and Pearson's correlation was assessed. We defined a significant correlation between a metabolic marker and an OTU if $P < 0.05$ after FDR correction. Similarly, the average intensity of the best markers from Prepuna species (*A. imbricata* and *H. doellii*) at 2870 and 3170 m.a.s.l was linked to OTU abundances from RSS samples collected at similar elevations (9 samples, 3 plant species, 2 elevations, Fig. S1). The most relevant bacterial families were defined by exploring the total number of related OTUs correlated to at least one metabolic marker. A literature search was carried out to develop hypotheses about the potential roles of these bacterial families. For this literature search, names of the most relevant bacterial families were screened on PubMed (<https://pubmed.ncbi.nlm.nih.gov/>) for established functional roles (e.g. plant growth-promoting bacteria), which were then reported to enrich the interpretation of this data.

3. Results

3.1. Influence of plant species and environment on rhizochemistry

Untargeted eco-metabolomics to assess rhizochemicals of four plant species and the associated rhizosphere collected at different elevations (Fig. 1 and Table S1) yielded 9008 features after pre-processing, from which 4629 were detected in rhizosphere samples and 4366 in both rhizosphere and aboveground samples (Table S4). As a first step, we focused on the metabolic features detected in both rhizosphere and plant samples. The PCA indicated that both (i) plant species and (ii) environmental factors influenced rhizochemicals (Fig. S2). Plant species were grouped according to their vegetation belt (i.e. Prepuna, *A. imbricata* and *H. doellii*, and Steppe species, *J. frigida* and *A. spinosissima*). Metabolic fingerprints were also influenced by elevation (Fig. S2).

3.2. Chemical diversity decreases under challenging conditions

Richness and Shannon's diversity of the rhizochemistry were dependent on plant species and elevation (Fig. S3). Prepuna species (*A. imbricata* and *H. doellii*) displayed fewer metabolic features as compared to Steppe species (*A. spinosissima* and *J. frigida*). Chemical richness decreased at the lowest sites, where hydric stress is higher for the species inhabiting these sites (Díaz et al., 2024; Dussarrat et al., 2022). The impact of elevation was also analysed at the species level (Fig. S4). The lowest richness scores were detected at 3770 and 4480 m. a.s.l in the rhizosphere of *J. frigida*, where richness levels were comparable to those observed in lowland species (Fig. S4). Unfortunately, *A. imbricata* was not sampled at its lowest and highest survival sites, explaining the distinct pattern for this species (Díaz et al., 2019). Within species, Shannon indices did not differ significantly (Figs. S3 and S4). Overall, these results indicate that plants or microbes tend to have a narrower chemical repertoire under stressful conditions (i.e. at lower and higher survival sites for each species).

3.3. Rhizochemistry predicts plant environment

In *J. frigida*, 216 features detected in both rhizosphere and plants were linked to elevation. Similarly, 8 features responded to elevation in *A. imbricata*, while 54 showed a significant trend. In comparison, 5 features detected in both rhizosphere and aerial parts of *A. spinosissima* were linked to elevation (Table S5). Rhizochemistry could be used to predict plant elevation, which was used as a proxy of the environment, from the three species with high accuracies (Fig. 2 and S5). However, it should be noted that the predictions for *A. spinosissima* refer to the classification score (since only two sites were collected for this species),

whereas quantitative predictions were made for the other species. The top metabolic predictors were ranked based on their coefficient in the models (Table S6). Results from models on permuted sets excluded the potential randomness of these predictions for each species (Fig. 2). Subsequently, the robustness of the predictive features (hereafter referred to as markers) was tested on *J. frigida*. Model equation developed on 2021 samples could efficiently predict the elevation of rhizosphere samples from *J. frigida* collected in April 2019 (Fig. 2). Importantly, the linear response of rhizochemicals under *J. frigida* was supported by the accurate prediction of rhizosphere samples collected at 4480 m.a.s.l, an elevation that was not present in the 2019 sample set.

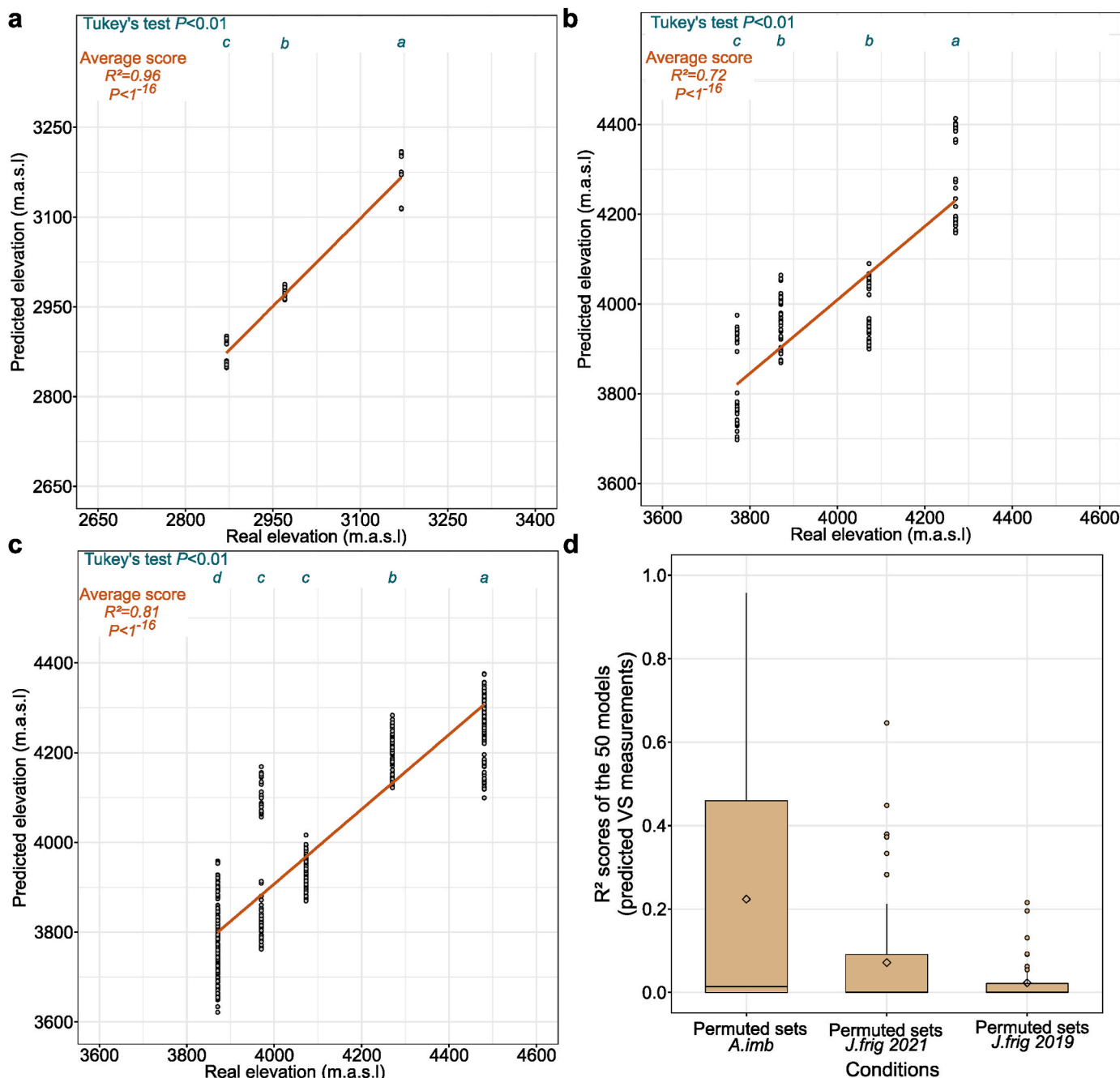


Fig. 2. | Prediction of elevation levels using metabolic fingerprints of rhizosphere samples. a. Partial least squares regression (PLSr) analyses using *A. imbricata* significant features (8 features). b. PLSr analyses using significant features (216 features) of rhizosphere samples collected under *J. frigida* in 2021. Model equation was developed on samples collected in 2021 and directly applied to 2019 rhizosphere samples (216 features). c. PLSr analyses using 50 permuted datasets. In boxplots, the diamond represents the mean while the solid dark line represents the median. PLSr models were performed 50 times. Tukey's tests were performed to compare predictions between elevation levels ($P < 0.01$).

Model predictions were validated using 50 permutation sets (Fig. S5).

Although some rhizosphere-specific features (*i.e.* features detected in soil samples exclusively) responded to elevation, predictions were not improved when adding these features to the PLSr models (Tables S7 and S8). Altogether, these results demonstrate that variations in environmental conditions induce a significant change in rhizochemistry, which could be used to deduce the altitude of the sample independently of the year of sampling.

3.4. Similarities in the response of rhizochemistry between species

Similar patterns were observed with the putative annotation of the significant metabolic markers between the different species. In the rhizosphere of *A. imbricata*, secondary (or specialised) metabolism was predominant (Fig. 3a and Table S9). Phenolics were the most widely represented chemical metabolite class. Terpenes, coumarins and cinnamic acids were also included among metabolic predictors, as well as some primary compounds such as organic acids. For *J. frigida* rhizosphere, putative annotations were balanced between primary and

secondary metabolisms (Fig. 3b). While phenolic pathways were prevalent again, terpenes were over-represented in this species. Within primary metabolism, a high proportion of the predictors referred to lipids (40 compounds), together with organic acids. Overall, similarities and differences were observed in the rhizosphere response to elevation depending on the plant species under which rhizosphere samples were collected.

To test the convergence degree, the elevation of rhizosphere samples from *H. doellii* and *A. spinosissima* was predicted using the equation developed on *A. imbricata* or *J. frigida*, respectively. The elevation of both species was considerably predictable with accurate predictions of *H. doellii* elevation (delta between real and predicted elevation of less than a hundred metres) and a significant distinction between the two elevation levels collected for *A. spinosissima* (Fig. 4). This result indicates convergent chemical responses of the surrounding rhizosphere to the elevation gradient between species.

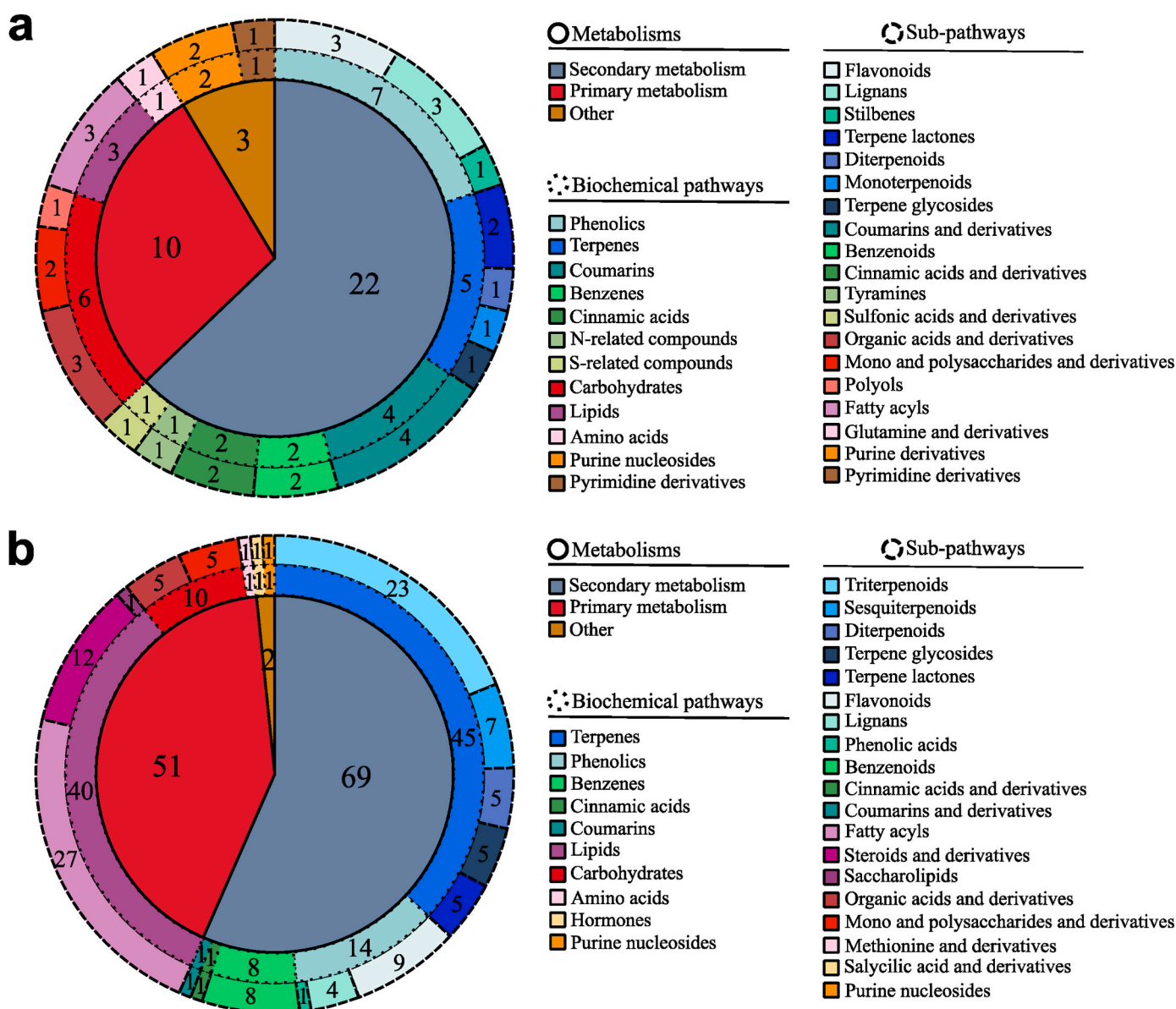


Fig. 3. | Annotation of the metabolic markers. a. Annotation of elevation markers from *A. imbricata*. b. Annotation of elevation markers from *J. frigida*. Unknown metabolic features were not displayed in this figure. See Table S9 for more details.

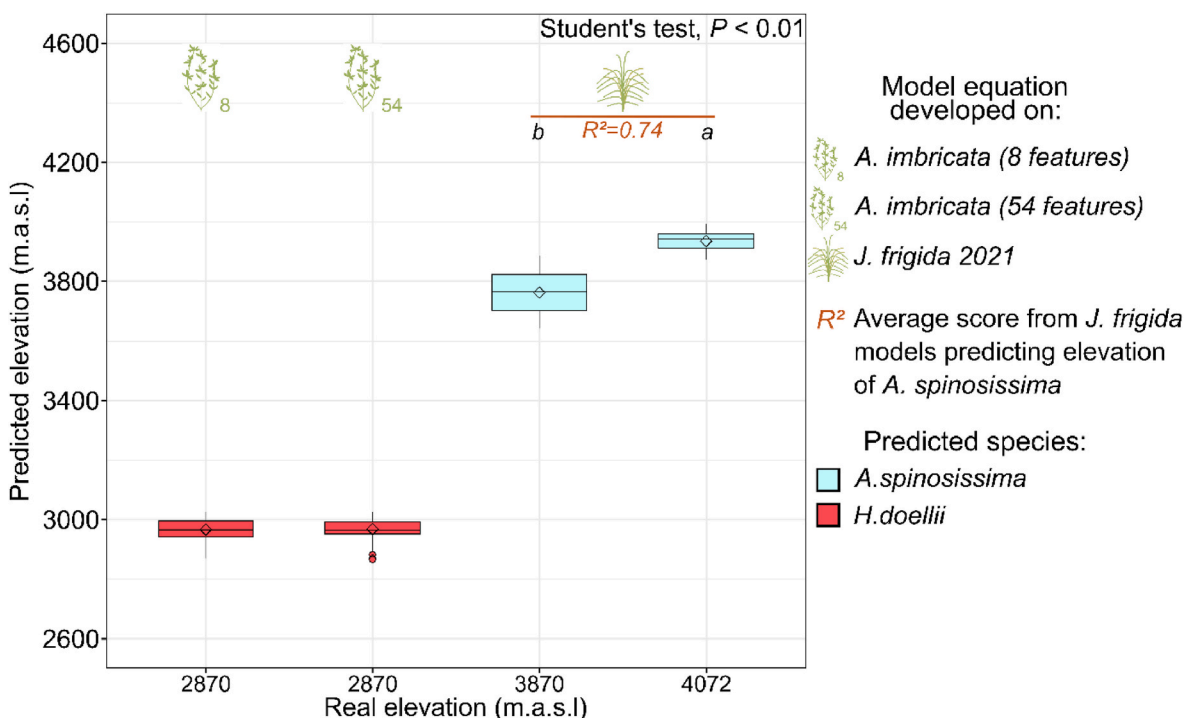


Fig. 4. | Prediction of elevation levels from independent conditions using metabolic markers of *A. imbricata* or *J. frigida*. Partial least squares regression analyses (PLSr) to predict elevation levels of *H. doellii* and *A. spinosissima*, respectively. Model equations were developed on *A. imbricata* (8 or 54 features) or *J. frigida* and directly applied to the independent samples. PLSr models were performed 50 times. Student's test was performed to compare predictions between elevation levels ($P < 0.01$). In boxplots, the diamond represents the mean while the solid dark line represents the median.

3.5. Convergences are also found in the response of bacterial communities to the elevation gradient

Next, we checked whether OTU abundances could be used to predict elevation levels independently of the species under which rhizosphere samples were collected (Fig. S1). Variation in OTU abundances could predict elevation levels of all species with significant distinctions between Prepuna, Puna and Steppe levels (Fig. S6). "Real" predictions (*i.e.* predicting elevation of 4 species previously excluded from the dataset) yielded high accuracies. The top 1% predictive OTUs (*i.e.* 105) allowed for similar predictive scores and models were validated using permuted datasets (Fig. 5a, Table S10). From these 105 OTUs, the most represented taxon positively correlated to elevation levels was Gaiellaceae. In contrast, Rubrobacteraceae and Cytophagaceae were the most negatively correlated taxa. In addition, some bacterial families such as Bradyrhizobiaceae were either positively or negatively correlated to elevation (Fig. 5b). A functional analysis was then performed on the most predictive OTUs to depict the underlying putative KEGG Orthology (ko) pathways (Table S11). Large overlaps were observed between the annotation of the most predictive OTUs and the predictive rhizochemicals. For instance, pathways linked to nitrogen metabolism, synthesis of precursors (*e.g.* amino acids) and secondary metabolism (*e.g.* terpenoids) were observed in both analyses, while others were overrepresented (*e.g.* pentose phosphate pathway) in OTU analyses (Table S12). The 1% most predictive OTUs represented a great proportion of the most correlated OTUs in a previously developed network of bacterial interactions in the Atacama transect (*i.e.* highest degree centrality and drivers, Mandakovic et al., 2023), highlighting their significance in the dynamics of bacterial communities (Table S13). Besides, the positive ratios of positive:negative connections (*i.e.* 1.7 and 3.8 for Steppe and Prepuna, respectively) suggested a need for cooperation at the most extreme elevations, which correspond to the most stressful environments (Table S13). Altogether, these results shed light on the existence and significance of chemical and ecological (*i.e.* dynamics of bacterial communities) convergences

belowground in response to the elevation gradient.

3.6. Rhizochemical markers are linked to the response of aboveground metabolism and bacterial communities

We tested the relationship between the rhizosphere predictive metabolites (*i.e.* markers) and both aboveground metabolism and soil bacterial communities (Fig. S1). A total of 19 (32% of *A. imbricata* markers) and 64 (30% of *J. frigida* markers) rhizosphere markers were also linked to elevation in aboveground samples from one to three plant species (Fig. 6a, Table S14). Without FDR correction, these numbers reached 63% and 58% for *A. imbricata* and *J. frigida*, respectively (Table S15). These metabolic predictors belonged to carbohydrate, phenolic and terpene pathways (*e.g.* succinic and glutamic acids, catechins). In addition, strong correlations were observed between OTU and the intensity of the best metabolic markers (Fig. 6). A total of 111 OTU relative abundances were correlated with the average intensity of at least one *J. frigida* rhizosphere marker, including 34 bacterial families. Similarly, 184 OTUs were correlated with *A. imbricata* rhizosphere markers, which included 42 bacterial families (Tables S16 and S17). Great overlaps were observed with 21 bacterial families, each containing at least one related OTU correlated with a metabolic marker (Fig. S7). In addition, these families strongly referred to the top 1% predictive OTUs used to predict elevation levels. A literature survey showed that bacterial families that correlated with chemical markers in both Prepuna and Steppe species were associated with potential effects on nitrogen and phosphorous cycle while bacteria linked to plant growth, metal detoxification and salinity (*e.g.* Geodermatophilaceae, Caulobacteraceae, Micrococcaceae) were more overrepresented in Prepuna (Fig. 6b and c and Table S17 for references).

Finally, we extracted the OTU abundances from bulk soil (BS) samples to investigate correlations between chemical markers and OTU abundances (Fig. S1). A total of 647 and 14 BS OTUs were correlated with at least one metabolic marker from *J. frigida* and *A. imbricata*

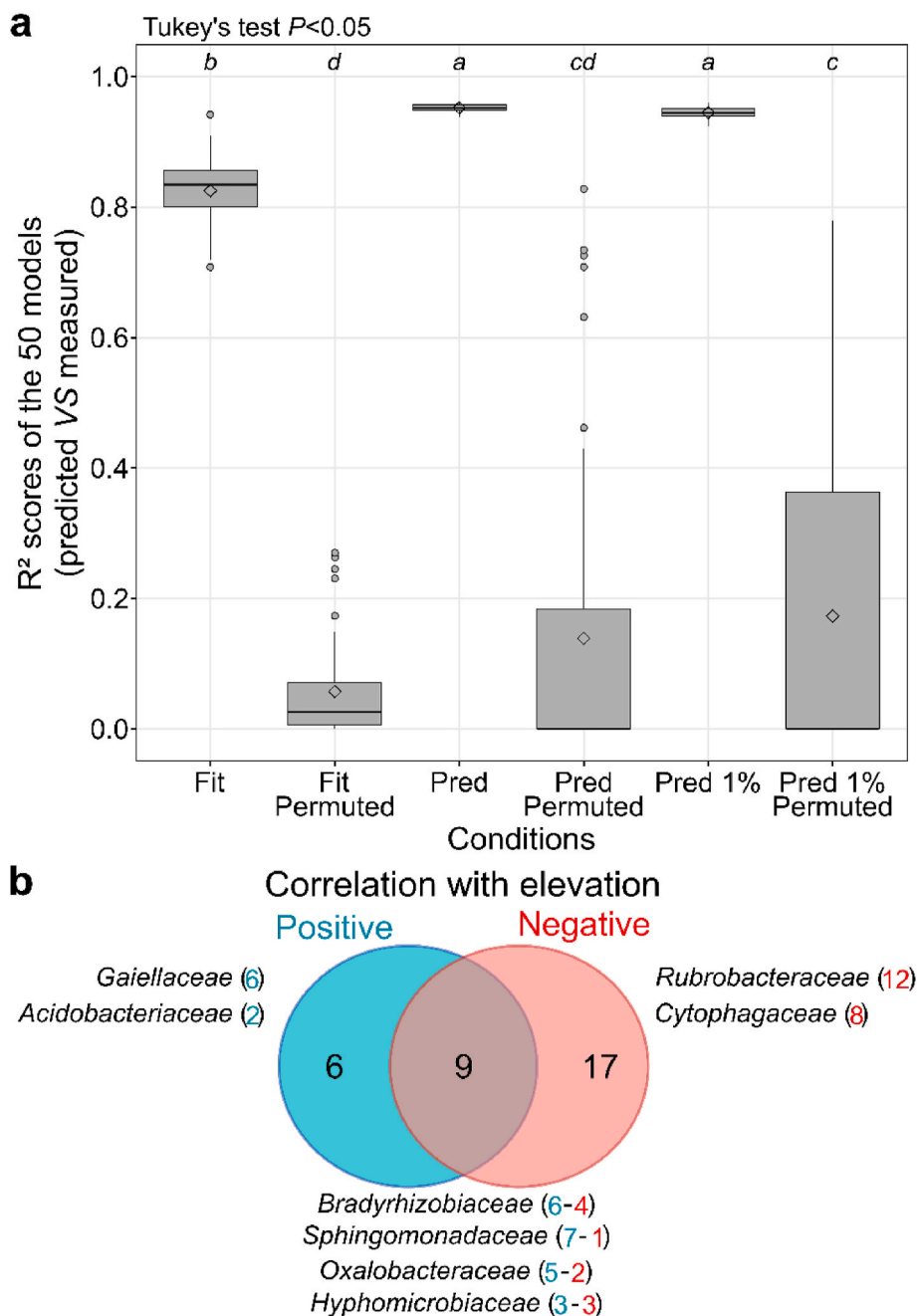


Fig. 5. | Response of soil bacterial communities to the elevation gradient. **a.** Predictive capacity of PLSr models using significant OTUs (*Fit/Pred* models, 1973 OTUs) or the top 1% most predictive OTUs (*Pred 1%* models, 105 OTUs) to predict the elevation of rhizosphere samples. All samples were used for “Fit” models. For predictive models (*Pred*), four species were excluded from the dataset (*i.e.* testing set). Model equation was developed on the remaining samples and directly applied to the testing set. Fifty permuted datasets were developed for each modelling condition. Tukey’s tests were performed to compare predictions between elevation levels ($P < 0.05$). **b.** Venn diagram of the bacterial families involved in the top 1% predictive OTUs. Numbers in parentheses refer to the number of OTUs included in the corresponding bacterial family. While examples are provided, more details are provided in [Table S10](#).

rhizosphere samples, respectively ([Table S18](#)). To test whether diffusion of the rhizochemical response could explain such differences, we tried to predict the elevation level of soil samples in open areas collected near *A. imbricata* or *J. frigida*. While *J. frigida* rhizosphere could be used to predict the elevation of samples collected in open areas with 64% accuracy, a similar trend was not observed when using *A. imbricata* rhizosphere ([Fig. S8](#)). Overall, combining metabolome and bacterial community predictive analyses highlights that (i) rhizochemistry captures environmental variation in extreme natural ecosystems, (ii) convergent and divergent chemical responses exist between plant species and environments, and (iii) these responses are potentially

associated with the dynamics of soil bacterial communities.

4. Discussion

4.1. Rhizochemistry is tailored to natural stress gradients

Here, we applied predictive metabolomics on rhizosphere samples under four plant species in the extreme Atacama Desert to uncover that rhizochemistry was tailored to environmental constraints. First, variation in chemodiversity indices was observed, supporting the role of abiotic parameters in shaping rhizochemistry ([Jansson and Hofmockel,](#)

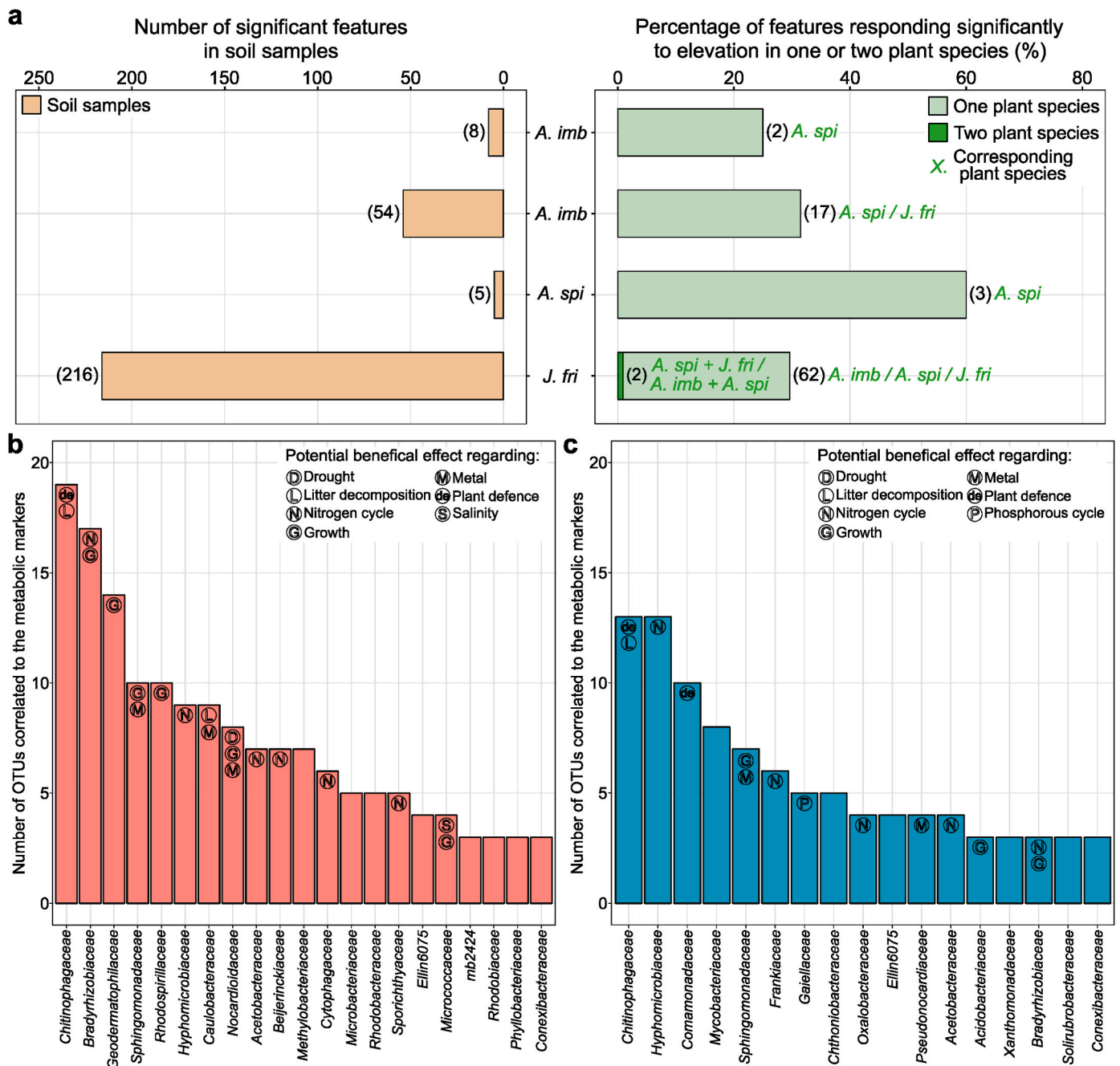


Fig. 6. | Correlations between rhizosphere metabolic predictors and plant aboveground response and soil bacterial communities. **a.** Numbers in brackets indicate the number of significant features in rhizosphere or plant samples for a given condition. All reported plant features were significant using Tukey’s test ($P < 0.05$, FDR correction). *A. imb*: *A. imbricata*, *A. spi*: *A. spinosissima*, *J. fri*: *J. frigida*. **b-c.** Correlated bacterial families to the best metabolic markers ($P < 0.05$, FDR correction, Pearson’s correlation) from *A. imbricata* in the Prepuna (**b**) or *J. frigida* in the Steppe (**c**). For ease of view, bacterial families were presented only if at least three related OTUs were significantly correlated. Additional information in [Tables S16 and S17](#).

2020). Richness and Shannon’s diversity were lower at low elevations, which coincided with the lifespan variation, with mostly annual plants inhabiting the Prepuna (2400–3300 m.a.s.l) and perennials the Steppe (4000–4500 m.a.s.l). These results are consistent with the effect of abiotic constraints on chemodiversity indices (Eshel et al., 2021; Kleine and Müller, 2014) and illustrate the stress gradient faced by Atacama species. In addition, plants negatively modulate the richness at lower altitude limits when environmental pressure becomes critical, potentially to limit the energy-consuming synthesis of secondary metabolites. However, while reducing their chemical repertoire, plants must maintain the regulation of specific compounds to thrive in these extreme

altitudes, which should allow for predictions of elevations based on rhizochemistry. This hypothesis was supported by the ability of several rhizosphere metabolites to predict the elevations of *A. imbricata* and *J. frigida* with 96 or 74% accuracy, respectively. Although great accuracy was observed for both species, the difference in predictive capacity (74 against 96%) might be due to (i) a lower sample size for *A. imbricata* and (ii) the difference in lifespan. As *J. frigida* is a perennial plant, potential variation in terms of age could lead to higher chemical variation and therefore lower global prediction accuracy. However, one limitation of our metabolomic approach to the rhizosphere is the inability to distinguish precisely between the plant or edaphic origin of the detected

metabolites. Given the universality of the metabolism, it is difficult, if not impossible, to separate the two compartments (Allwood et al., 2021). Although only tedious labelling experiments could provide this precision level, most of the rhizochemicals reported also responded significantly in the aerial parts, suggesting a plant origin.

4.2. Complementary metabolic strategies in below- and aboveground tissues can help face harsh climatic and edaphic constraints

Most rhizosphere metabolic markers predicting plant environment in the present study were linked to nitrogen and phosphorous cycles, salt excess or water deprivation, as well as bacterial communities. For instance, markers included allantoin, guanosine and glutamic acid, which relate to the nitrogen cycle and purine degradation pathways (Izaguirre-Mayoral et al., 2018; Kaur et al., 2021). Organic acids (e.g. succinic acid), including ursolic acid, were previously linked to soil quality and salt stress mitigation (Carvalhais et al., 2011; Long et al., 2020). Terpenes, flavonoids, coumarins and cinnamic acids were found in samples from both vegetation belts, in agreement with their functions at the plant-microbe interface (Hassan and Mathesius, 2012; Vismans et al., 2022). Additional markers, such as lipids, were mainly observed in the rhizosphere of *J. frigida*. Lipids are considered key drivers of plant-microbe interactions (Siebers et al., 2016). A consequent portion of these rhizosphere markers also responded to elevation in aboveground plant material. These observations suggest that many of the rhizosphere markers were likely the result of plant responses to the environment rather than microorganism-derived processes. Overall, these results are complementary to previous studies. For example, transcriptomic analysis of 32 Atacama species identified positively selected genes closely linked to resource acquisition (Eshel et al., 2021). In contrast to our rhizosphere analysis, a metabolomics study on leaves of Atacama plants uncovered compounds protective against extreme temperature, solar irradiance and drought, but very few metabolites linked to nutrient deprivation (Dussarrat et al., 2022). Hence, the survival of Atacama plants may lie in their ability to adapt an organ-specific metabolic strategy to cope with severe climatic and edaphic constraints, in coordination with their bacterial communities.

4.3. Convergence in metabolic and ecological adaptation to the extreme environmental gradient

While certain markers differed between vegetation belts (e.g. lipids), our results suggest a certain convergence in adaptations to the environment in Steppe or Prepuna species. In addition, the rhizosphere markers predicting the environment of *J. frigida* or *A. imbricata* were able to substantially predict the elevation of *A. spinosissima* (74% accuracy) and *H. doellii*, respectively. Our results indicated that convergences might be found belowground. This finding is consistent with the discovery of metabolic convergence in aerial plant parts in response to abiotic threats, although previous studies have shown a strong phylogenetic signal in rhizochemistry (Dussarrat et al., 2022; Eshel et al., 2021; Walker et al., 2022; Wandrag et al., 2020). These convergences might also be explained by the fact that root and root exudate metabolic profiles tend to present less variation between species when compared to aerial plant parts (McLaughlin et al., 2023). The analysis of OTU abundances also provided insights into the existence of convergent patterns in the dynamics of bacterial communities across the elevation gradient. In line with the fact that extreme lands are recognised as hotspots for adaptive convergence (Xu et al., 2020), our results suggest that these events may not be restricted to aerial tissues. While this discovery represents a major advance in the field, further analyses comparing the response of rhizochemistry under a wider range of species would be needed to support this hypothesis.

Apart from this key finding in the context of evolutionary biology, it should be noted that our predictive modelling approach is particularly well-suited to revealing convergent strategies. In contrast, previous

studies on extreme lands were mostly limited to comparing one agronomic species to a related extreme species, yielding the discovery of protective compounds highly specific to the species studied (Dussarrat et al., 2021). Using our methodology may help reveal additional convergences in other extreme lands. The next thrilling question will be to compare these convergences between extreme ecosystems and assess their similarities and divergences.

4.4. Chemical rhizosphere markers are linked to growth-promoting bacteria

Rhizosphere markers predicting plant elevation were correlated with OTU abundances, highlighting the influence of rhizochemicals on soil bacterial communities (Cotton et al., 2019; Del Valle et al., 2020; Philippot et al., 2013). These results also support the place of rhizochemistry as a driver of ecosystem dynamics (Angulo et al., 2022; Chung, 2023; Van Nuland et al., 2017). OTU of the Chitinophagaceae were the most correlated bacterial family in both Prepuna and Steppe environments, reflecting the need to facilitate litter decomposition and defence against soil pathogens (Carrión et al., 2019; Xu et al., 2021). Hyphomicrobiaceae, Bradyrhizobiaceae and Acetobacteraceae, which are known to cope with nitrogen starvation, were observed in both vegetation zones as well as Gaiellaceae and Sphingomonadaceae, bacteria associated with phosphorous limitations or metal pollution, respectively, and already detected in other areas of the Atacama Desert (Sultana et al., 2023; Wang et al., 2022; Bartholomäus et al., 2024). Thus, although a certain level of divergence was noticed in the chemical rhizosphere markers between plant species, similar ecological consequences on the bacterial communities were found. However, in the Prepuna, more correlations with growth-promoting bacteria were found, such as Geodermatophilaceae (Bartholomäus et al., 2024). These growth-promoting bacteria may be beneficial for annual species mostly found at low altitudes (*versus* perennials in the Steppe), as they may promote faster development to escape periods of drought (Díaz et al., 2019). In addition, some of the correlated bacterial families have been shown to relate to plant metabolism, which is supported by our analytical approach. For instance, coumarins and organic acids have been associated with Oxalobacteraceae, Comamonadaceae and pathogen taxa, respectively (Del Valle et al., 2020; Wen et al., 2020; Yu et al., 2021). Flavonoids were shown to mediate interactions with nitrogen fixers such as Oxalobacteraceae (Hong et al., 2022), a connection also observed in the present study. Finally, intriguing results arose from the analysis of soil samples collected in open areas. Richness and Shannon's diversity were high in soil collected in the Steppe and metabolic markers from *J. frigida* rhizospheres could partially predict the elevation of samples from open areas, while *A. imbricata* rhizosphere markers failed. The abundance of rhizochemicals from *J. frigida* was also correlated to changes in OTU abundances in open areas of the Steppe. Perennial Poaceae, such as *J. frigida*, can develop vegetative runners belowground, which may explain a diffusion of root chemicals. The implications of this diffusion and its relation with soil bacterial communities on Atacama dynamics need further investigation, especially since an influence of plant-soil feedback on heterospecific plant successors has been shown (Wilschut et al., 2023).

In addition, our results identify predictive markers in terms of rhizochemistry and soil bacterial communities that can be used as a tool for diagnosing soil quality in the context of climate change. A previous study highlighted that leaf metabolites used by Atacama plants to cope with extreme climate were also detected in agronomic species (Dussarrat et al., 2022). As rhizosphere compounds and chemical families responding to the extreme gradient were also shared between plant families, they represent an additional layer of interest for crop engineering and intercropping.

5. Conclusion

Working with wild species in natural environments, particularly in an extreme habitat such as the Atacama Desert, is challenging. Moreover, these species are not easily grown or reproduced under laboratory conditions. However, field studies can provide key biological insights that are not achievable under laboratory conditions. This work provides unexpected insights into the response of rhizochemistry to environmental constraints: direct (*i.e.* similar markers), as well as indirect (*i.e.* similar consequences on bacterial communities) convergences, could be observed between plant species. Rhizochemistry predicted plant environment consistently across years and, within a vegetation belt, chemical markers from one plant species could be used to predict the elevation level of other species. Convergences were also observed when analysing the variation of bacterial communities across an elevation gradient, thus providing major insights in the context of ecological and evolutionary biology. Results also showed that our approach offered the possibility of unravelling generic compounds that hold great promise to improve crop performances or diagnose soil quality under abiotic pressure. Finally, predictive metabolomics combined with OTU abundance analyses highlighted the diffusion of the rhizosphere markers of *J. frigida* and its implications for bacterial communities. Rhizochemistry and its influence on conspecific and heterospecific neighbours deserve greater attention to better predict ecosystem dynamics in the Atacama Desert and other extreme ecosystems.

CRedit authorship contribution statement

Thomas Dussarrat: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Claudio Latorre:** Writing – review & editing, Visualization, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Millena C. Barros Santos:** Writing – review & editing, Investigation. **Constanza Aguado-Norese:** Writing – review & editing, Writing – original draft, Visualization, Resources, Investigation, Formal analysis. **Sylvain Prigent:** Writing – review & editing, Visualization, Formal analysis, Data curation. **Francisca P. Díaz:** Writing – review & editing, Resources, Funding acquisition, Conceptualization. **Dominique Rolin:** Writing – review & editing, Methodology, Conceptualization. **Mauricio González:** Writing – review & editing, Writing – original draft, Visualization, Resources, Methodology, Investigation, Data curation. **Caroline Müller:** Writing – review & editing, Visualization, Validation, Investigation, Funding acquisition. **Rodrigo A. Gutiérrez:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. **Pierre Pétriaccq:** Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Data availability

Data and metadata are included in supplemental tables and were deposited on MassIVE (MSV000093102, <https://doi.org/10.25345/C5JM23S4X>), which include raw spectra, non-normalised dataset, and feature metadata in the sections “raw”, “other” and “metadata”, respectively. Sample metadata are available in [Supplemental Table S1](#). Illumina sequence data were previously deposited on the National Center for Biotechnology Information (BioProject, accession number PRJNA489888) ([Mandakovic et al., 2023](#)).

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2024.109662>.

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