Metabolic responses of the isopod *Porcellionides pruinosus* to nickel exposure assessed by ^{1}H NMR metabolomics

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A B S T R A C T

This work aimed at characterizing the metabolome of the isopod *Porcellionides pruinosus* and at assessing its variations over 14 days under laboratory culture conditions and upon exposure to the contaminant metal Nickel (Ni). The spectral profiles obtained by ^{1}H NMR spectroscopy were thoroughly assigned and subjected to multivariate analysis in order to highlight consistent changes. Over 50 metabolites could be identified, providing considerable new knowledge on the metabolome of these model organisms. Several metabolites changed non-linearly with Ni dose and exposure time, showing distinct variation patterns for initial (4 days) and later time points (7 and 14 days). In particular, at day 4, several amino acids were increased and sugars were decreased (compared to controls), whereas these variations were inverted for longer exposure, possibly reflecting earlier and more intensive moulting. Other variations, namely in betaines and choline-containing compounds, were suggested to relate with osmoregulation and detoxification mechanisms. Ni also had a marked effect on several nucleotides (increased upon exposure) and a moderate impact on lipids (decreased upon exposure). Overall, this study has provided new information on the Ni-induced metabolic adaptations of the *P. pruinosus* isopod, paving the way for improved mechanistic understanding of how these model organisms handle soil contamination.

Significance: This study provided, for the first time to our knowledge, a detailed picture of the NMR-detectable metabolome of terrestrial isopods and of its fluctuations in time and upon exposure to the contaminant metal Nickel. Several time- and dose-dependent changes were highlighted, providing mechanistic insight into how these important model organisms handle Ni contamination.

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1. Introduction

While traditional tests in ecotoxicology provide important information about the general impact on the individual (e.g. survival or reproduction) and the population, the thorough understanding of how a contaminant affects an organism requires the study of different levels of biological organization. Metabolic profiling (metabolomics) enables alterations in the cellular metabolome (i.e. inventory of endogenous small molecules <1 kDa) to be monitored, potentially revealing marker signatures of exposure and providing new insights on induced biochemical events [1]. Even though the use of metabolomics in environmental sciences is still scarce, some studies have addressed the changes in the metabolic profile of some key model species (e.g. [2–4]). Regarding soil ecotoxicology, most studies have used earthworms (e.g. [5,6]), and, to our knowledge, there is only one report on terrestrial isopods, which presented metabolite fingerprints for the

species *Porcellio scaber* and *Oniscus asellus* [7], in a healthy (non-exposed) condition.

Terrestrial isopods are macro-invertebrates involved in decomposition processes, vegetal litter fragmentation and recycling of nutrients [8–13], therefore being essential to maintain the function and structure of the soil compartment. Exposure to xenobiotics may affect edaphic organisms, consequently changing the overall soil function, and decreasing soil quality and soil services [14]. The species *Porcellionides pruinosus* has been described as a good test-organism to evaluate soil contamination or changes in its habitat and several endpoints, from the individual to lower organizational levels, have been used to evaluate the effects caused by different stressors [15–19].

Nickel (Ni) is a naturally occurring metal in the environment and it is considered an essential trace element for diverse biotic functions in organisms. However, due to its high usage in industry and the involuntary anthropogenic release, this metal can reach high concentrations in soils [20], potentially disturbing the ecosystems' homeostasis. In previous studies using cell lines [21], daphnids [22] or fish [23], Ni toxicity has been related to oxidative stress, a process commonly

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induced by metals, which reflects the imbalance between the production of reactive pro-oxidant species and the ability to neutralize their harmful effects. Moreover, Ni is considered a carcinogenic metal, as it has been reported to impact gene transcription and translation processes, and even the phosphate cycle [12,24]. Regarding Ni effects on soil invertebrates, the information available is still very scarce, being focused on bioaccumulation and mortality testing (e.g. [20, 25]). Recently, our group has addressed the response of terrestrial isopods to long-term Ni exposure by measuring changes in energy reserves and the activity of detoxification enzymes [12]. This study confirmed the induction of oxidative stress and further revealed that other modes of action should be responsible for Ni toxicity, thus calling for a more detailed understanding of Ni effects at the molecular level.

The aim of the present work is to explore the potential of NMR metabolomics for characterizing the metabolome of terrestrial isopods of the species *Porcellionides pruinosus*, and for assessing its fluctuations over 14 days under laboratory culture conditions and upon Ni exposure. Two doses have been selected for this study: a low dose corresponding to the maximum allowed Ni concentration in the Canadian framework guideline (50 mg Ni/kg soil) [26], and a high dose corresponding to $5 \times$ this concentration (250 mg Ni/kg soil). New insights into time- and dose-dependent effects of Ni on the isopods metabolism are expected to emerge and to provide a more thorough understanding of biological responses to this metal.

2. Materials and methods

2.1. Test organisms and culture procedure

Organisms used in this assay belong to the species *Porcellionides pruinosus* Brandt (1833), and were previously collected from a horse manure heap and maintained for several generations in laboratory cultures. In culture, isopods were fed ad libitum with alder leaves (*Alnus glutinosa*) and maintained at 22 ± 1 °C, with a 16:8 h (light:dark) photoperiod. Twice a week cultures were water sprayed and food was provided. Only adult organisms (15–25 mg wet weight) were used in the experiments and no distinction between genders was made, although pregnant females were excluded. Organisms with abnormalities or moulting characteristics were also excluded from the trials.

2.2. Soil spiking

LUFA 2.2 soil (LUFA-Speyer 2.2, Germany) is a sandy loam and was used for the exposures. This soil presented a total organic carbon content of $1.77 \pm 0.2\%$, a pH of 5.5 ± 0.2, nitrogen content $0.17 \pm 0.02\%$, texture characterized by $7.3 \pm 1.2\%$ clay, $13.8 \pm 2.7\%$ silt and $78.9 \pm 3.5\%$ sand, and a water-holding capacity (WHC) of $41.8 \pm 3\%$ (g/100 g). Soil was spiked with Nickel (Ni) at concentrations of 50 and 250 mg Ni/kg soil, with a final moisture content equivalent to 50% of the soil WHC. The concentration of 50 mg Ni/kg soil represented the maximum concentration allowed by the Canadian framework guideline [26].

2.3. Exposure experiments

Toxicity tests were performed in plastic boxes (26 length × 18 width × 7.5 height cm), containing approx. 2 cm height of LUFA 2.2 soil layer, with 40 isopods (15–25 mg each) per box. Alder leaf discs (Ø 10 mm, ± 20 mg) were supplied as food, using a quantity that prevented organisms to remain on top and avoid contaminated soil. Organisms were exposed to control soil, 50 mg and 250 mg Ni/kg soil, in a 16:8 h (light:dark) photoperiod, at 20 °C, for 14 days. During this period, organisms were sampled at four time points: prior to exposure (named further on as time 0 or day 0), 4 days, 7 days and 14 days after exposure.

In each sampling time, and for each treatment, three replicates of 6 organisms each were collected.

2.4. Sample preparation for NMR

Organisms were weighted and stored at −80 °C for a period no longer than one month. Before analysis, each sample (6 organisms per replicate) was homogenized using a sonicator (Kika Labortechnik, V200Scontrol, Germany) in 600 µL of K-Phosphate/D_2O 0.1 M buffer, pH 7.0 and centrifuged (10.000 rpm, 10 min, 4 °C). Then, 400 µL of supernatant were transferred into a 5 mm NMR tube to which 100 µL of D_2O (to provide a lock signal) containing 0.1% TSP-d4 (used for shimming) were added.

2.5. NMR data acquisition and processing

NMR spectra were recorded on a Bruker Avance DRX 500 spectrometer operating at 500.13 MHz for ^1H observation at 300 K. Standard 1D spectra with water presaturation (pulse programme 'noesypr1d', Bruker library) were acquired with a 6510 Hz spectral width, 32 k data points, a 2 s relaxation delay (d1), 100 ms mixing time (d8) and 256 scans. All 1D spectra were processed with a 0.3 Hz line broadening, zero filling to 64 k data points, manual phasing and baseline correction. The chemical shifts were referenced internally to the glucose signal at δ 5.23 ppm. 2D ^1H-^1H total correlation (TOCSY) spectra, ^1H-^{13}C heteronuclear single quantum correlation (HSQC) and *J*-resolved spectra were also registered for selected samples to assist spectral assignment. The main acquisition and processing parameters for these experiments are provided in Supplementary Table S1.

2.6. Multivariate analysis

After normalization by total spectral area and scaling to Unit Variance (UV), principal component analysis (PCA) and partial-least squares discriminant analysis (PLS-DA) were applied to the 1D spectra (suppressed water region excluded) using the SIMCA-P 11.5 software (Umetrics, Umeå, Sweden). A default seven-fold internal cross validation was used, from which Q^2 and R^2 values, respectively reflecting predictive capability and explained variance, were extracted. The results were visualized through scores scatter plots and corresponding loadings, which were recovered by multiplying the loading weights *w* by the standard deviation, and coloured as a function of variable importance in the projection (VIP) in Matlab 7.14.0.739 (The MathWorks Inc., Massachusetts, USA). Hierarchical cluster analysis (HCA) was also applied, based on the Pearson correlation coefficient with single linkage, using the GENE-E software (http://www.broadinstitute.org/cancer/software/GENE-E/index.html). Moreover, unidimensional statistical total correlation spectroscopy (STOCSY) [27] was performed in Matlab 7.14.0.739 for assignment and search of metabolic correlations.

2.7. Spectral integration and univariate statistical analysis

To evaluate metabolite quantitative variations, selected signals in the 1D spectrum were integrated using Amix-Viewer (version 3.9.14, BrukerBiospin, Rheinstetten, Germany) and normalized by the total spectral area. The resulting data was plotted into a heatmap using the GENE-E software (http://www.broadinstitute.org/cancer/software/GENE-E/index.html). For each metabolite, the difference between the means of two groups (control and exposed) was considered significant when the *p*-value, calculated using the two-sample *t* test or the nonparametric analogue Wilcoxon rank sum test with continuity correction, was lower than 0.05 (confidence level 95%).
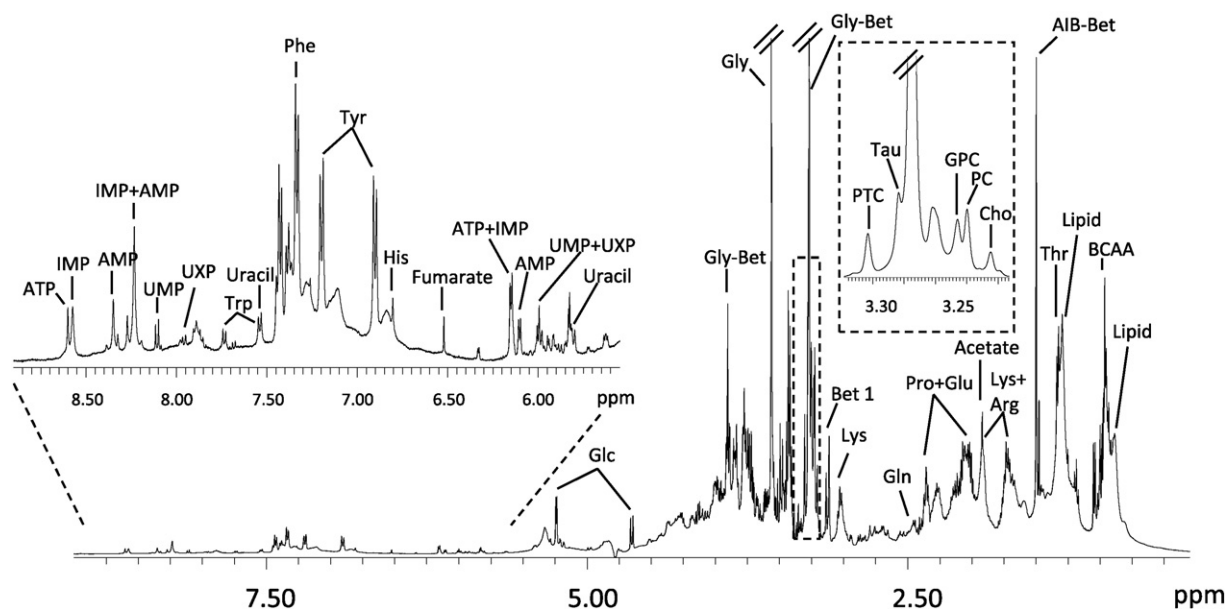
Fig. 1. Representative 500 MHz ¹H NMR spectrum of the aqueous supernatant obtained from the terrestrial isopod species *Porcellionides pruinosus*. Some assignments are indicated: three-letter code used for amino acids, ADP adenosine diphosphate, AIB-Bet 2-aminoisobutyrate-betaine, AMP adenosine monophosphate, ATP adenosine triphosphate, BCAA branched chain amino acids (leu, ile, val), Bet betaine, Cho choline, Glc glucose, Gly-Bet glycine-betaine, GPC glycerophosphocholine, IMP inosine monophosphate, PC phosphocholine, PTC phosphatidyl-choline, Tau taurine, UMP uridine monophosphate, UTP uridine triphosphate, UXP (UDP/UTP).

3. Results

3.1. Metabolic profile of the species P. pruinosus

Fig. 1 shows a typical ¹H NMR spectrum of an isopod supernatant, where a multitude of signals was detected, reflecting the complex sample composition. Spectral assignment strongly relied on 2D NMR spectra, namely ¹H-¹H TOCSY, ¹H-¹³C HSQC and *J*-resolved spectra, shown in Supplementary Figure S1, and matching of 1D and 2D spectral data to reference spectra in the BBIOREFCODE-2–0–0 database (Bruker Biospin, Rheinstetten, Germany), as well as other existing databases [28,29]. The low-frequency region (δ 0–3) shows resonances from organic acids (e.g. lactate, acetate, succinate, citrate, 2-ketoglutarate) and from several amino acids, both common (e.g. branched chain amino acids, arginine, lysine, proline, glutamate, glutamine) and less common (e.g. 3-aminobutyrate, 4-aminobutyrate, 2-aminoisobutyrate). Moreover, several spin systems with patterns slightly shifted from those of free amino acids were detected in the TOCSY spectrum (correlations with the δ 4.1–4.4 α-CH region) and assigned to amino acid residues bound in a peptide chain. These compounds were partially responsible for some broad signals observed in the 1D profile, together with lipid resonances. In particular, lipid fatty acyl chain signals were clearly detected in the TOCSY and HSQC spectra and further highlighted in the 1D STOCSY correlation plot (Supplementary Figure S2A). Moreover, cross peaks typical of glyceryl resonances in phospholipids (δ 4.09, 4.30, 5.22) as well as of CH₂-N and CH₂-OP choline protons (δ 3.88, 4.46) were detected in the TOCSY spectrum, thus suggesting phophatidylcholines to be the main lipids present. In the mid-frequency region (δ 3–5.5), additional metabolites detected included glucose, some unassigned sugars, taurine, choline-containing compounds and betaines, with the most abundant betaine being glycine-betaine. Another relatively prominent betaine detected was assigned to 2-aminoisobutyrate-betaine, based on the correlation highlighted in the 1D STOCSY between the δ 1.50 and the 3.26 singlets (Supplementary Figure S2B). In the high-frequency region (δ 5.5–10), the signals detected were assigned mainly to aromatic amino acids (tyrosine, phenylalanine, tryptophan), fumarate, nitrogenated bases and nucleotides (AMP, ADP, ATP, IMP, UMP, uracil and UDP/UTP), some of these assignments having been confirmed by spiking with standard compounds. Overall, as summarized in Supplementary Table S2, over 50 metabolites together with lipids and small peptides could be detected, providing, for the first time to our knowledge, a detailed picture of the metabolic profile of *P. pruinosus* terrestrial isopod.

3.2. Metabolic variations in control organisms over time

In order to evaluate the inherent biological variability of the organisms used and assess the possible influence of sampling time on the metabolic responses to Ni exposure, the metabolic fluctuations in control animals upon culture time (0 to 14 days) were investigated. The scores scatter plot resulting from applying PCA to the spectral profiles (Fig. 2A) showed a trend for separation in PC2 between samples collected at days 0 and 4 and those collected at later time points, although one of the time 0 samples was further away from its group. Hierarchical cluster analysis (HCA) corroborated these results, as shown by the dendogram in Fig. 2B. Spectral integration of individual metabolites was then carried out to assess the quantitative variations responsible for the observed clustering. The main variations are summarized in the form of a heatmap shown in Fig. 2C, which is colour-coded according to the percentage of variation in the controls collected at 4, 7 and 14 days relatively to the time 0 controls. While the levels of some metabolites were found to be stable over time (e.g. arginine, proline, fumarate, phosphocholine) or to vary randomly (e.g. lipids), other compounds showed quite consistent variations over culture time. Interestingly, several amino acids (e.g. branched chain and aromatic amino acids) showed a common variation pattern, non-linear with time, decreasing in the first 4 days and increasing at 7 and 14 days. Betaines increased from early days, this increase being more marked at day 4 for the most abundant glycine-betaine, whereas the levels of choline and glycerophosphocholine (GPC) were decreased (at day 4 and at all time points, respectively). Another particularly prominent variation was the increase in adenosine triphosphate (ATP), accompanied by the decrease in inosine monophosphate (IMP). The nitrogenated base uracil and the nucleotide uridine monophosphate (UMP) were also found to be increased in relation to time 0. Finally, in regard to carbohydrates and organic acids, glucose and several sugars were decreased at days 7 and/or 14, whereas succinate was increased.
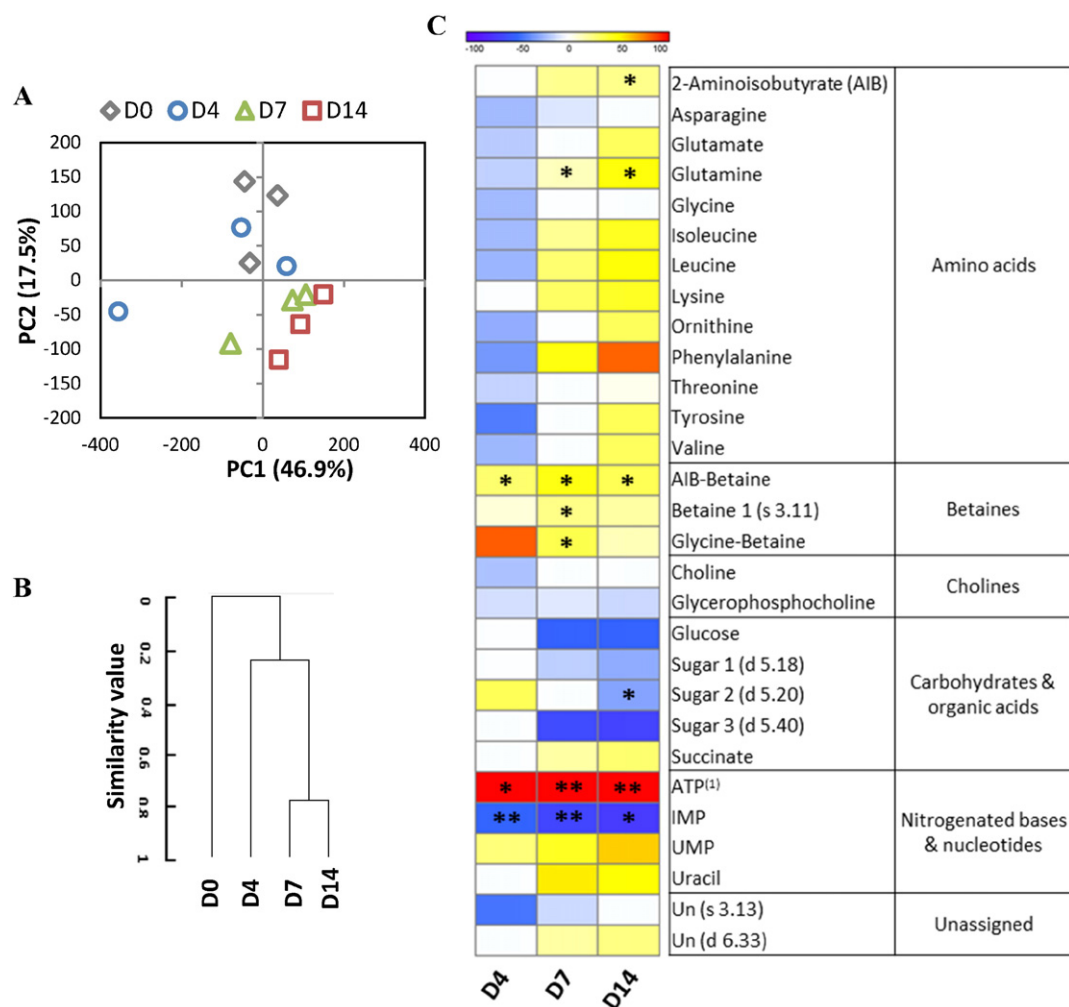
Fig. 2. (A) Scores scatter plot obtained by PCA of ^{1}H NMR spectra from control isopods collected at different culture times (0, 4, 7 and 14 days); (B) Dendogram resulting from applying HCA to the same spectral matrix as in (A); (C) Heatmap of main metabolite variations in control isopods over time. The colour scale reflects the direction and magnitude of these variations (%) in relation to time 0. * $p < 0.05$; ** $p < 0.01$. (1) Variation higher than 100%.

3.3. Nickel-induced metabolic variations

As a first approach to unveil trends and clusters within samples collected in the Ni exposure assay, PCA and HCA have been applied to the spectral matrix comprising controls and isopods exposed for 4, 7 or 14 days, the resulting scores scatter plot and dendogram being shown, respectively, in Supplementary Figure S3A,B. As it is apparent from those plots, when considering all time points, control samples did not cluster together nor were separated from exposed ones. A major factor accounting for this scattered distribution could be the sampling time since it was found to significantly influence the isopods metabolome, as described in the previous section. Therefore, multivariate analysis was repeated considering a sub-set of spectra for each exposure period. The respective PCA and PLS-DA scores scatter plots are shown in Fig. 3. Control and exposed samples showed a trend for separation by PCA and a reasonable discrimination by PLS-DA (Q^2 0.4–0.5 for 2 latent variables). Inspection of the corresponding LV1 loadings colourcoded as a function of variable importance in the projection (VIP) (Fig. 4), allowed a number of signals with VIP > 1, representative of specific metabolites, to be selected for integration, in order to assess the magnitude and statistical significance of the variations. The results were expressed as the percentage of increase or decrease in low- and high-dose Ni-exposed isopods relatively to controls, and summarized in the form of a heatmap (Fig. 5). A number of amino acids, which had already shown a typical variation pattern over culture time, increased

in the first 4 days of Ni exposure and then showed decreased levels, compared to controls, at longer exposure periods. Betaines showed mostly an increasing trend, with 2-aminoisobutyrate betaine increasing at earlier exposure times and glycine-betaine at later exposure times. In regard to choline-containing compounds, choline was increased by day 4 (both doses) and day 7 (high dose), while PC and GPC showed increasing trends for longer exposures at high dose. Glucose and other unknown sugars were decreased after the first 4 days of exposure but then increased their relative levels, especially after 14 days. Nucleotides were significantly increased at specific doses and time points; In particular, AMP and IMP were increased at 14 days of exposure to low Ni concentration, while ATP and UMP were increased at 7 days of high dose exposure. Finally, non-significant decreases were observed for lipid resonances, namely at day 4 for the low dose and at days 7 and 14 for the high dose.

Although many of the changes did not reach statistical significance, these results clearly show that Ni caused the metabolome of isopods to change in a time- and dose-dependent manner. For most metabolites, the pattern of variations upon 4 days of exposure was clearly distinct from that characterizing the longer exposure periods. Dose-dependency was also noticed, although the stronger effects did not necessarily occur at the higher dose. HCA performed on the percentage of variation in relation to controls corroborated these observations, as a first node separated day 4 from days 7 + 14, irrespectively of dose, and a second node separated the days 7 + 14 cluster into low and high dose (Supplementary Figure S3C).
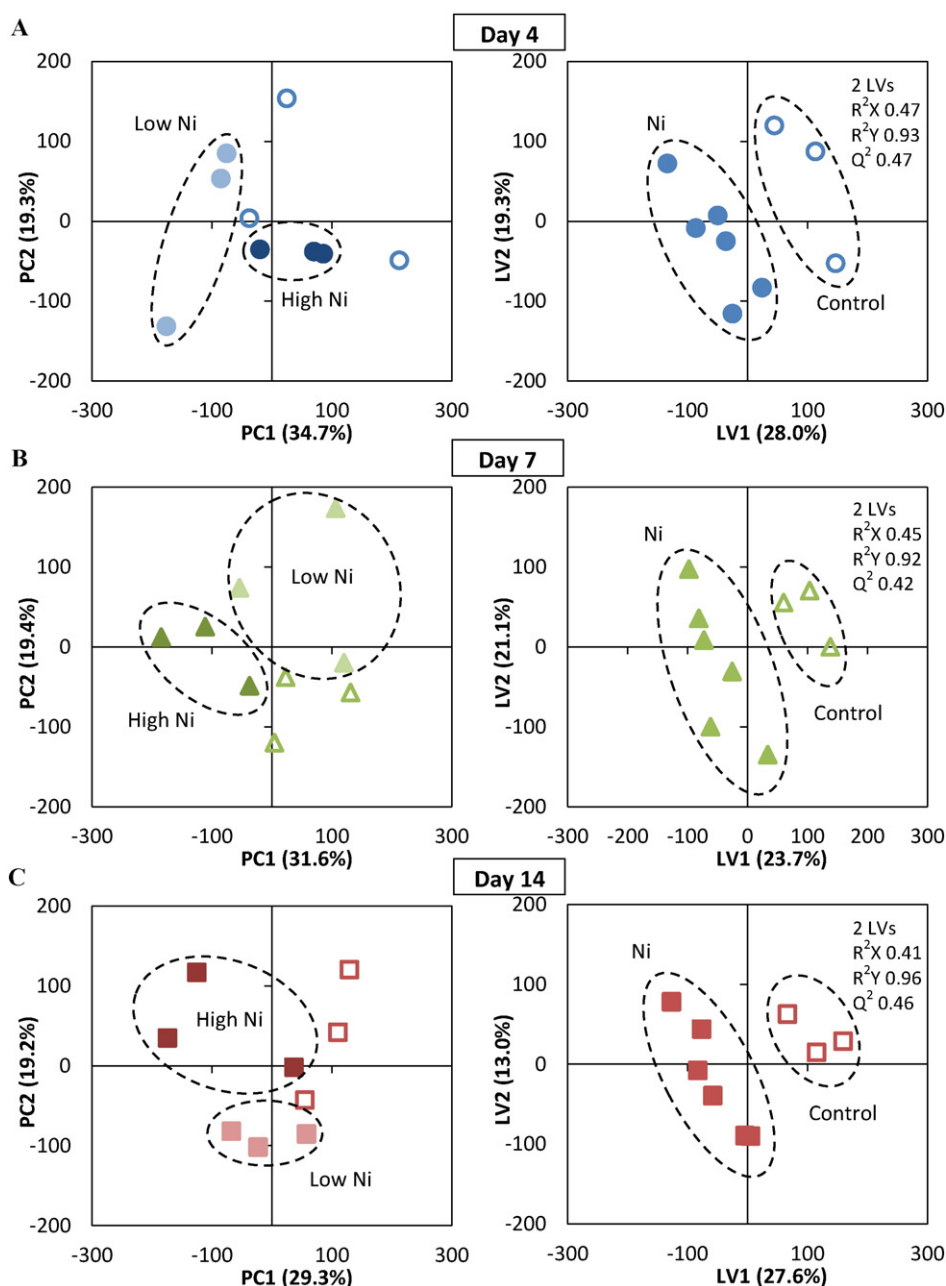
Fig. 3. Scores scatter plots obtained by PCA (left) and PLS-DA (right) of ¹H NMR spectra from control isopods and isopods exposed to Ni for (A) 4 days, (B) 7 days and (C) 14 days.

4. Discussion

In this work, the metabolic composition of the isopod species *P. pruinosus* was thoroughly characterized by 1D and 2D NMR spectroscopy, which allowed establishing an important background for subsequent studies addressing the changes in the isopods metabolome upon different stimuli. Several amino acids, organic acids, betaines, choline-containing compounds, sugars and nucleotides were identified, adding a significant amount of new information to the metabolic composition previously reported for these organisms [7].

Interestingly, the metabolome of control organisms suffered several changes along the culture time, the variations found at day 4 being often distinct from those recorded for days 7 and 14, particularly in regard to amino acids and sugars. While amino acids decreased after the initial 4 days, an increase was observed for longer periods, which was accompanied by a decrease in glucose and other sugars and an increase in succinate, one of the intermediates of the tricarboxylic acid (TCA) cycle. It is

thus possible that these concerted changes at later times reflect the production of amino acids from carbohydrates to sustain isopods growth. In regard to the marked difference in amino acids variation between day 4 and subsequent time points, it is possible that it reflects an initial adaptation of the organisms to the new environment. Indeed, terrestrial isopods are considered sociable [30] and known to produce chemical clues to interact and aggregate [18,31]. These social interactions have been highlighted to affect the organisms' fitness according to group size, as described by the Allee effect, whereby isolated organisms tend to spend more time and energy searching for other individuals with which to aggregate [32]. Therefore, the change from culture boxes where approximately 100 organisms (from mancae to juveniles and adults) co-habited, to boxes with a total of 20 organisms (all adults) could possibly alter the production of such chemical clues and, consequently, metabolite levels. Moreover, when organisms were moved to the test boxes, filled with "new" Lufa 2.2 soil, the structure and the microbiome of the soil was altered. Such alteration could also have
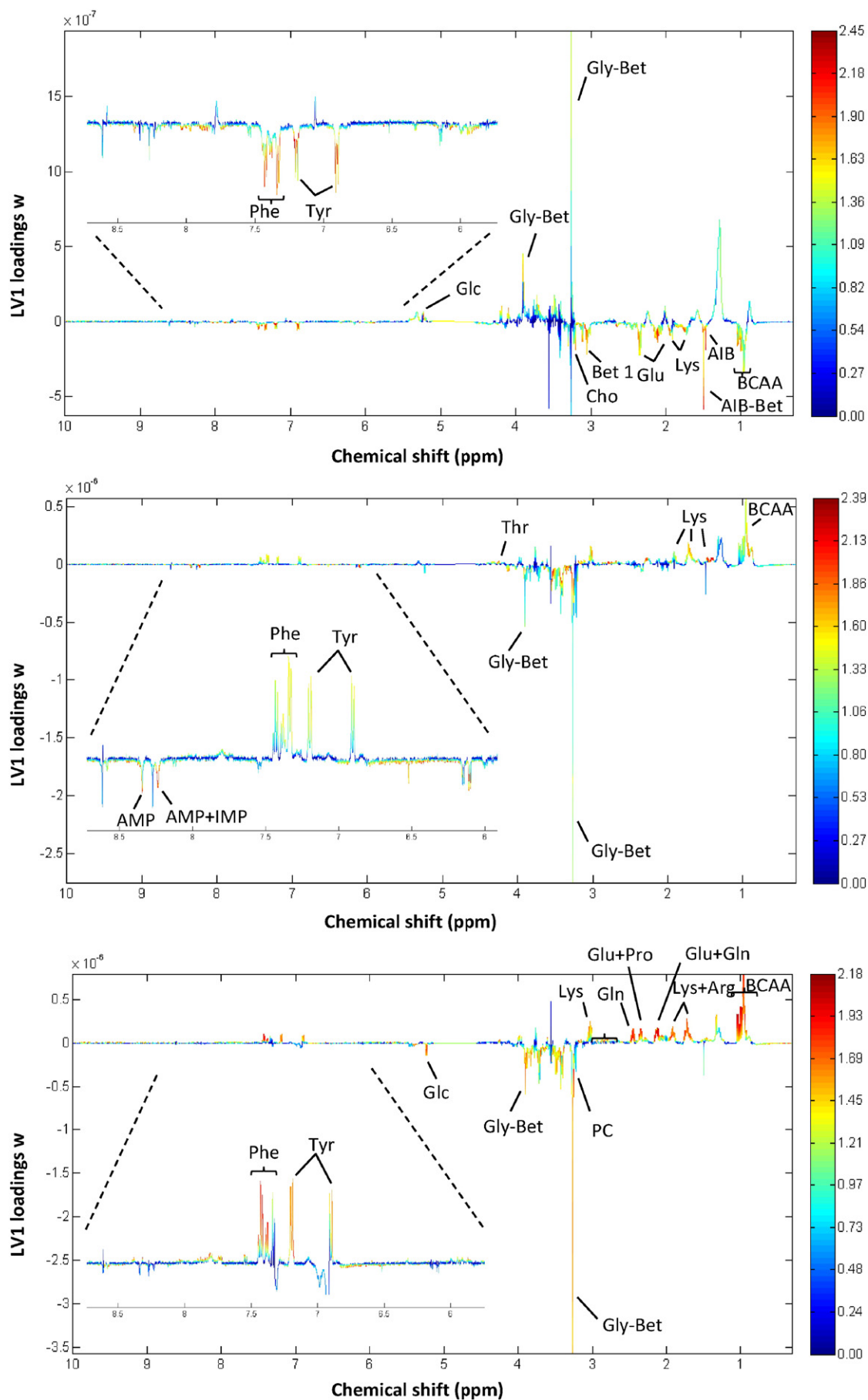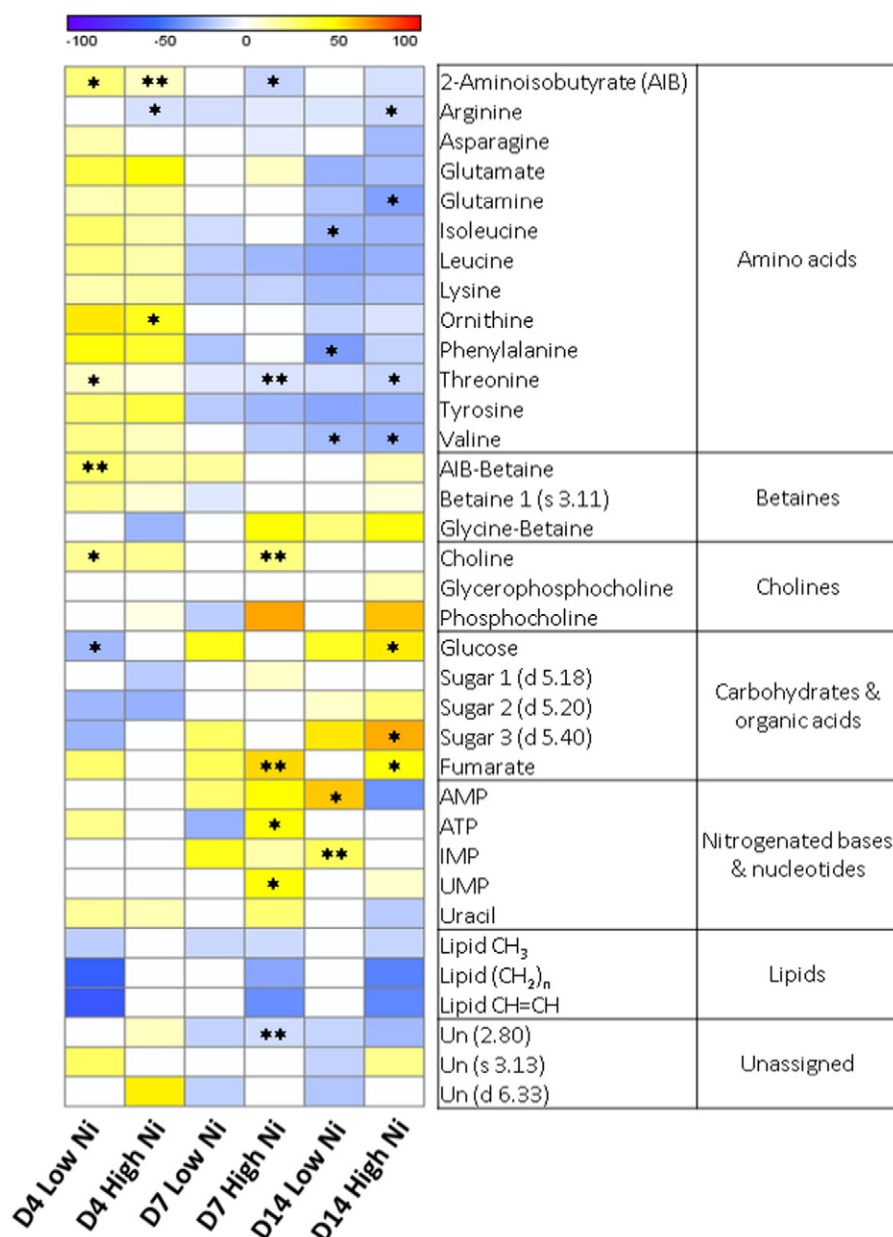
Fig. 5. Heatmap of main metabolite variations in Ni-exposed isopods (low dose 50 mg/kg soil; high dose 250 mg/kg soil), at different exposure periods (4, 7 and 14 days). The colour scale reflects the direction and magnitude of these variations (%) in relation to controls (collected for each respective time point). * $p < 0.05$; ** $p < 0.01$.

possibly affected the organisms' metabolome as the food available is expected to be differently colonized by microorganisms, thus altering the availability and acquaintance of essential or non-essential amino acids.

Another consistent variation in control isopods over time was the increase in betaines, namely glycine-betaine, 2-aminoisobutyrate-betaine and a third betaine, which could not be unambiguously identified. Betaines are trimethylammonium derivatives of amino acids, which have been previously reported to be present in high abundance in isopods [7], as well as in other terrestrial invertebrates such as earthworms [33], and are known to play a crucial role in the maintenance of osmotic balance [34]. They may be provided in the diet or produced from the oxidation of choline (which together with glycerophosphocholine were in fact found to decrease over time). Thus, the betaines' increase may possibly reflect osmoregulation mechanisms to facilitate the organisms'

adaptation to the soil composition and moisture. Finally, prominent changes were noted in the levels of nucleotides, particularly IMP and ATP. While the former was decreased in relation to time 0 controls, ATP and, to a lesser extent, UMP and uracil were increased over culture time, suggesting energy storage and altered nucleotide metabolism.

Regarding the metabolic consequences of Ni exposure, one of the most evident alterations was the increase in several amino acids after the first 4 days of exposure, followed by a consistent decrease to levels lower than the respective controls, at the subsequent time points evaluated (7 and 14 days). Several amino acids are known to be involved in moulting related processes [35], through multiple ways. For instance, branched chain amino acids (leucine, isoleucine and valine) are involved in muscle growth [36], as well as in haemocyanin synthesis and transportation [37], lysine is related to the production of elastin

Fig. 4. LV1 loadings *w*, coloured as a function of variable importance in the projection (VIP), corresponding to PLS-DA of ^{1}H NMR spectra from control isopods and isopods exposed to Ni for (A) 4 days, (B) 7 days and (C) 14 days. Some of the most important variations are labelled: three-letter code used for amino acids, AMP adenosine monophosphate, AIB 2-aminoisobutyrate, AIB-Bet 2-aminoisobutyrate-betaine, BCAA branched chain amino acids (leu, ile, val), Bet betaine, Glc glucose, Gly-Bet glycine-betaine, IMP inosine monophosphate, PC phosphocholine.

and collagen [38] and to the absorption of calcium for exoskeleton formation [39], phenylalanine and tyrosine are involved in the synthesis of the pigment melanin and in haemocyanin transportation processes [40], while glutamine is involved in muscle growth [41]. As in other crustaceans, growth and moult are tightly correlated in terrestrial isopods and dependent on the frequent replacement of their cuticle [39]. Although moulting is not considered the main excretion route of metals in these organisms [42], it has been reported to take part on this process. For instance, a previous study has shown that copper-containing granules were able to dissolve during the moult process allowing their excretion [43]. It has also been reported that the regular 28 days moulting cycle of isopods (species *Porcellio scaber*) could be altered by metal exposure [44]. In particular, organisms exposed to zinc-contaminated food showed a trend to moult earlier, as well as a higher number of moults. In that study, only approximately 20% of control organisms moulted in the first week of exposure, while this number increased to 50% in the case of animals exposed high zinc concentration (10 mg Zn/g food) [44]. Therefore, it is possible that the amino acids variation pattern observed in the present study may reflect a tendency for the organisms to anticipate moulting, although this was not confirmed by visual monitoring of moult processes.

Betaines showed a trend to increase upon Ni exposure, which, as already mentioned, may relate to their osmoprotective role. Moreover, their involvement in detoxification reactions through the transport and donation of methyl groups may also account for the observed variation. Choline levels were increased compared to controls, especially at days 4 and 7. Choline may result from the hydrolysis of acetylcholine, a neurotransmission mediator, through the action of the enzyme acetylcholinesterase (AChE) in the synaptic cleft [45,46]. Indeed, in a previous study on the same isopod species, we have found AChE activity to be elevated upon Ni exposure [12], possibly to prevent acetylcholine over-accumulation, which would lead to over-stimulation of cholinergic receptors and disruption of the nervous system function [47]. Furthermore, choline and phosphocholine are important constituents of cell membrane phospholipids and their variation may also relate to membrane degradation processes. The trend for lipid resonances to decrease in Ni-exposed isopods also agrees with this hypothesis.

In regard to carbohydrates, the variations in glucose and other sugars may relate to their utilization for the production of chitin to support early and more intensive moulting. Fumarate, which is a TCA cycle intermediate and part of the purine nucleotide and the ornithine-urea cycles involved in nitrogen excretion, was also found to increase, especially at later time points and high doses. The impact on nucleotide metabolism was also apparent from the significant changes in monophosphate nucleosides (AMP, UMP, IMP) and ATP. Concordantly, Ni has been previously reported to interfere with nucleotide synthesis and DNA replication in tumour cells [24].

Overall, this study has provided new information on how terrestrial isopods adapt their metabolism upon exposure to the contaminant metal Ni. In particular, the metabolome was found to change in a dynamic, non-linear way with time and dose, showing, for most metabolites, distinct patterns between the initial and later time points tested (4 and 7/14 days). Interestingly, the mortality recorded in a parallel experiment using the same time points and doses [12] showed a similar number of dead animals in control and exposed conditions at day 4, but a significant increase in mortality upon Ni exposure for 14 days. It is thus likely that the metabolic profile characterizing the short-term (4 days) response to Ni reflects the ability of terrestrial isopods to cope with soil contamination. This agrees with other studies demonstrating isopods' high plasticity to adapt to the presence of metals, by storing them in granules and making them non-bioavailable [48,49]. Then, at longer exposure periods, Ni-induced toxicity was observed, as expressed by the increase in the number of dead animals, and the metabolic response pattern changed. Interestingly, most metabolites started to vary already after 7 days of exposure although the number of dead animals in the exposed setup was larger than in control conditions only

after 14 days [12], which clearly demonstrates the sensitivity of NMR metabolomics to detect early, subtle effects at the molecular level.

In summary, this work has allowed monitoring dynamic changes in the metabolome of the isopod species *P. pruinosus*, in control and Ni-exposure conditions, paving the way for improved mechanistic understanding of how these model organisms handle soil contamination.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jprot.2015.09.018.

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