We present proteome data from the microbiota (feces) after a diet shift from a natural diverse to a monocultural meadow with Dactylis glomerata. The abundant grasshopper species, Chorthippus dorsatus, was taken from the wild and kept in captivity and were fed with Dactylis glomerata for five days. For phytophagous insects, the efficiency of utilization of hemicellulose and cellulose depends on the gut microbiota. Shifts in environmental and management conditions alter the presence and abundance of plant species which may induce adaptations in the diversity of gut microbiota. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD005126.

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### Specifications Table

| Subject area                  | Biology |
|------------------------------|---------|
| More specific subject area   | Metaproteomics |
| Type of data                 | 1) Mass spectrometry data (*.raw) |
|                              | 2) Search output data (*.msf) |
|                              | 3) Figures (PowerPoint files) |
| How data was acquired        | Orbitrap Fusion mass spectrometer (Thermo Scientific) coupled with the TriVersa NanoMate (Advion Biosciences, Norwich, UK). |
| Data format                  | 1) msf (Proteome Discoverer output files) |
|                              | 2) pptx (PowerPoint files) |
| Experimental factors         | Microbial proteins were isolated from feces, proteolytically cleaved using trypsin and subsequently analyzed by LC-MS/MS |
| Experimental features        | 1) Grasshopper feces collection |
|                              | 2) Protein extraction |
|                              | 3) LC-MS/MS analysis |
| Data source location         | Leipzig, Saxony, Germany |
| Data accessibility           | Data is within this article. The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PRIDE: PXD005126. |

### Value of the data

- Protein assessment of the microbiota of the grasshopper species *Chorthippus dorsatus*.
- Metaproteome from the grasshoppers provides the basis for more functional analyses of the grasshopper microbiota.
- Relevant information for the grasshoppers ecology on the basis of its microbiota.

### 1. Data

We present the first dataset of this relevant type of phytophagous insects since there is so far no metaproteome dataset on the gut microbiota of grasshoppers available. To detect the diet-dependent metabolic adaptation, a shift in diet from a diverse food source to the single-species food *Dactylis glomerata* was performed.

### 2. Experimental design, materials and methods

#### 2.1. Grasshopper culture and feces sampling

We selected *Chorthippus dorsatus* that was at that time the most dominant grasshopper species on a ruderal meadow that has been under this type of land use for 20 years. The meadow was dominated by grasses, namely *Poa pratensis*, *Dactylis glomerata*, *Festuca pratensis*, and *Bromus sterilis*, which are all potential food plants for the generalist herbivores in grasslands [1–4]. Three days before the start of the experiment, we caught female and male grasshoppers with sweep nets and kept them separately in cages with a mixed grass diet from the meadow until the start of the experiment. In August 2014, 2 male and 2 female grasshoppers were transferred to a cage without food for 1 day to synchronize for gut content. On the following day *D. glomerata*, which was grown in a climate chamber under...
standardized and controlled soil and moisture conditions for about 2.5 months, was added to the cage. Excrements were sampled before (d0) and after adding *D. glomerata* at day 1, 3, and 5 (day 1, day 3 and day 5) for one week and frozen at −80 °C.

### 2.2. Protein extraction and sample preparation

After the sampling of feces (day 0, 1, 3, 5 and 6), three feces were pooled and were considered as one replicate. For protein extraction, to the feces 4 glass beads (3 mm, Carl Roth GmbH), two spatula tips of zirconium beads (0.1 mm diameter, Biospec.) and 800 μL of lysis buffer (500 mM NaCl, 50 mM...
Fig. 2. Phylogenetic resolution of identified proteins. (A) Stacked bar chart show the distribution of identified protein groups assigned to phylogenetic kingdoms. The two most abundant groups along the time points belong to kingdom Bacteria (in dark blue) and kingdom Plantae (in light blue). (B) Phylogenetic distribution at the phylum of Bacteria. 77% to 82% of all bacterial proteins were classified to phylum Firmicutes. The second most abundant phylum (range from 12% to 17%) was Proteobacteria. (C) Bar chart of bacterial alpha diversity over the time course. Error bars show the standard deviation between triplicate measurements. There were no significant differences of alpha diversity observed.
Tris–HCl, pH 8, 50 mM EDTA, 4% (w/v) SDS) were added. Feces were disrupted by FastPrep (3 × 1 min, 5.5 ms⁻¹, MP Biomedicals) and incubated for 15 min at 95 °C. After centrifugation (14,000 rpm, 5 min, 4 °C), the supernatant was taken. To the pellets 300 µL of lysis buffer were added and the procedure with FastPrep and heating was repeated exclusively for the pellet. The supernatant was precipitated over night at 20 °C with acetone (2.5 fold of ice-cold acetone). Protein pellets were harvested by centrifugation (14,000 rpm). Dried pellets were dissolved in 20 µL of SDS sample buffer (2% w/v SDS, 2 mM beta-mercaptoethanol, 4% v/v glycerol, 40 mM Tris–HCl pH 6.8, 0.01% w/v bromophenol blue), heated to 90 °C for 4 min and separated by SDS polyacrylamide gel electrophoresis. Proteins were stained in gel with Coomassie G-250 (Merck). Gel was cut into small pieces (band per samples), destained, dehydrated and proteolytically cleaved overnight at 37 °C trypsin (Promega) [5]. Extracted peptides were desalted using C18 ZipTip column (Merck Millipore). Peptide lysates were re-suspended in 0.1% formic acid and injected to liquid chromatography mass spectrometry (LC-MS/MS).

2.3. Mass spectrometric measurement and data analysis

Mass spectrometry was performed using Orbitrap Fusion (Thermo Fisher Scientific) coupled to a TriVersa NanoMate (Advion) as described [6]. To assess the protein functions of the different bacterial
community members, we assigned the identified proteins to clusters of orthologous groups (COGs). A principal component analysis (PCA) was applied as classical means of dimensionality reduction and visualization of multivariate data. PCA was assessed of log-transformed and normalized protein abundance profiles along the time scale (Figs. 1–5).
Fig. 5. Relative protein abundance levels. Protein abundances of selected proteins assigned for (A) Bacilli and (B) Clostridia were calculated based on the normalized spectral abundance factor (NSAF) and plotted along the time-line in order to observe species abundance changes in respect to their functional classification.

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References

[1] J.A. Vizcaino, E.W. Deutsch, R. Wang, A. Csordás, F. Reisinger, D. Rios, J.A. Dianes, Z. Sun, T. Farrah, N. Bandeira, P.A. Binz, I. Xenarios, M. Eisenacher, G. Mayer, L. Gatto, A. Campos, R.J. Chalkley, H.J. Kraus, J.P. Albar, S. Martinez-Bartolome, R. Apweiler, G.S. Omer, L. Martens, A.R. Jones, H. Hermjakob. ProteomeXchange provides globally coordinated proteomics data submission and dissemination, Nat. Biotechnol. 32 (3) (2014) 223–226.

[2] A. Franzke, S.B. Unsicker, J. Specht, G. Köhler, W.W. Weisser. Being a generalist herbivore in a diverse world: how do diets from different grasslands influence food plant selection and fitness of the grasshopper Chorthippus parallelus? Ecol. Entomol. 35 (2010) 126–138.

[3] S. Ingrisch and G. Köhler. Die heuschrecken Mitteleuropas, 1998.

[4] G. Köhler, F. Fritzlar, Fauna der Heuschrecken (Ensifera et Caelifera) des Freistaates Thüringen, Thüringer Landesanstalt für Umwelt und Geologie, 2001.
[5] N. Jehmlich, F. Schmidt, M. Hartwich, M. von Bergen, H.H. Richnow, C. Vogt, Incorporation of carbon and nitrogen atoms into proteins measured by protein-based stable isotope probing (Protein-SIP), Rapid Commun. Mass Spectrom. 22 (18) (2008) 2889–2897.

[6] V. Lünsmann, U. Kappelmeyer, R. Benndorf, P.M. Martinez-Lavanchy, A. Taubert, L. Adrian, M. Duarte, D.H. Pieper, M. von Bergen, J.A. Muller, H.J. Heipieper, N. Jehmlich, In-situ Protein-SIP highlights Burkholderiaceae as key players degrading toluene by para ring hydroxylation in a constructed wetland model, Environ. Microbiol 18 (4) (2016) 1176–1186.